

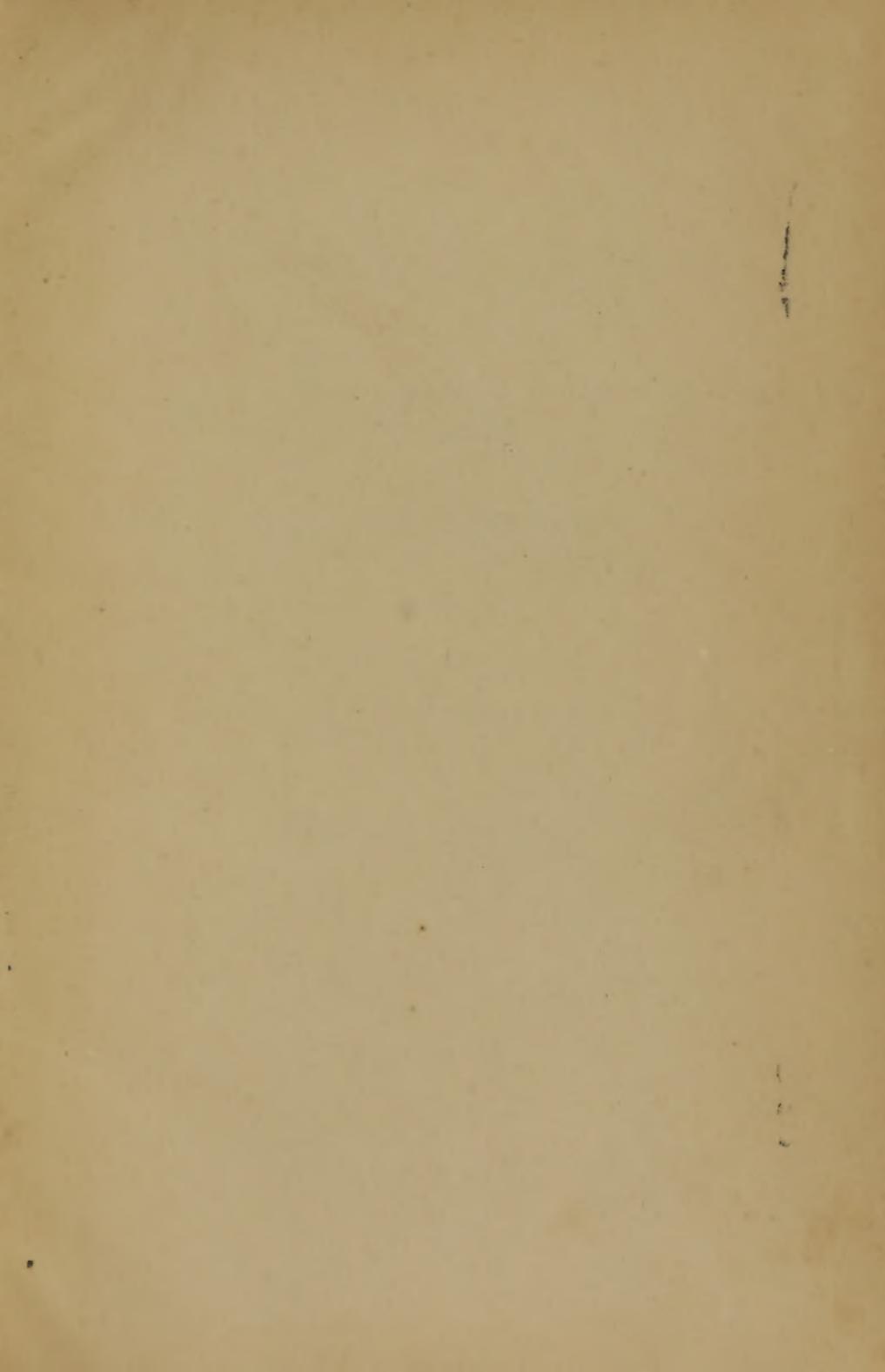
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SECTION-CUTTING:

A PRACTICAL GUIDE

TO THE

PREPARATION AND MOUNTING OF SECTIONS FOR
THE MICROSCOPE,

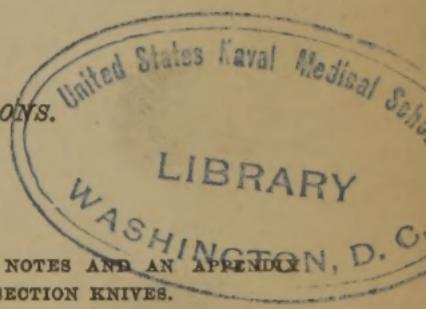
*SPECIAL PROMINENCE BEING GIVEN TO THE
SUBJECT OF ANIMAL SECTIONS.*

BY

DR. SYLVESTER MARSH.

WITH ILLUSTRATIONS.

REPRINTED FROM THE LONDON EDITION, WITH NOTES AND AN APPENDIX
ON THE SELECTION AND CARE OF SECTION KNIVES.



NEW YORK:
INDUSTRIAL PUBLICATION COMPANY.

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P R E F A C E.

If we glance at any of the numerous magazines devoted either wholly or in part to the subject of microscopy, we shall hardly fail to be struck with the numerous queries relating to SECTION-CUTTING, which are to be found in its pages. A simple explanation of this wide-spread want is afforded by the fact that the use of the microscope has at the present day extended to (and is still rapidly spreading amongst) vast numbers of students, who, in many instances, possess neither the leisure nor the means to refer for information to large and expensive textbooks. Moreover, were they actually to consult such works, they would practically fail to obtain the information of which they are in need, for the coveted instruction is to be found in those treatises only in a scattered and fragmentary form—no work with which we are acquainted treating of the subject in anything like a detailed manner. To fill this

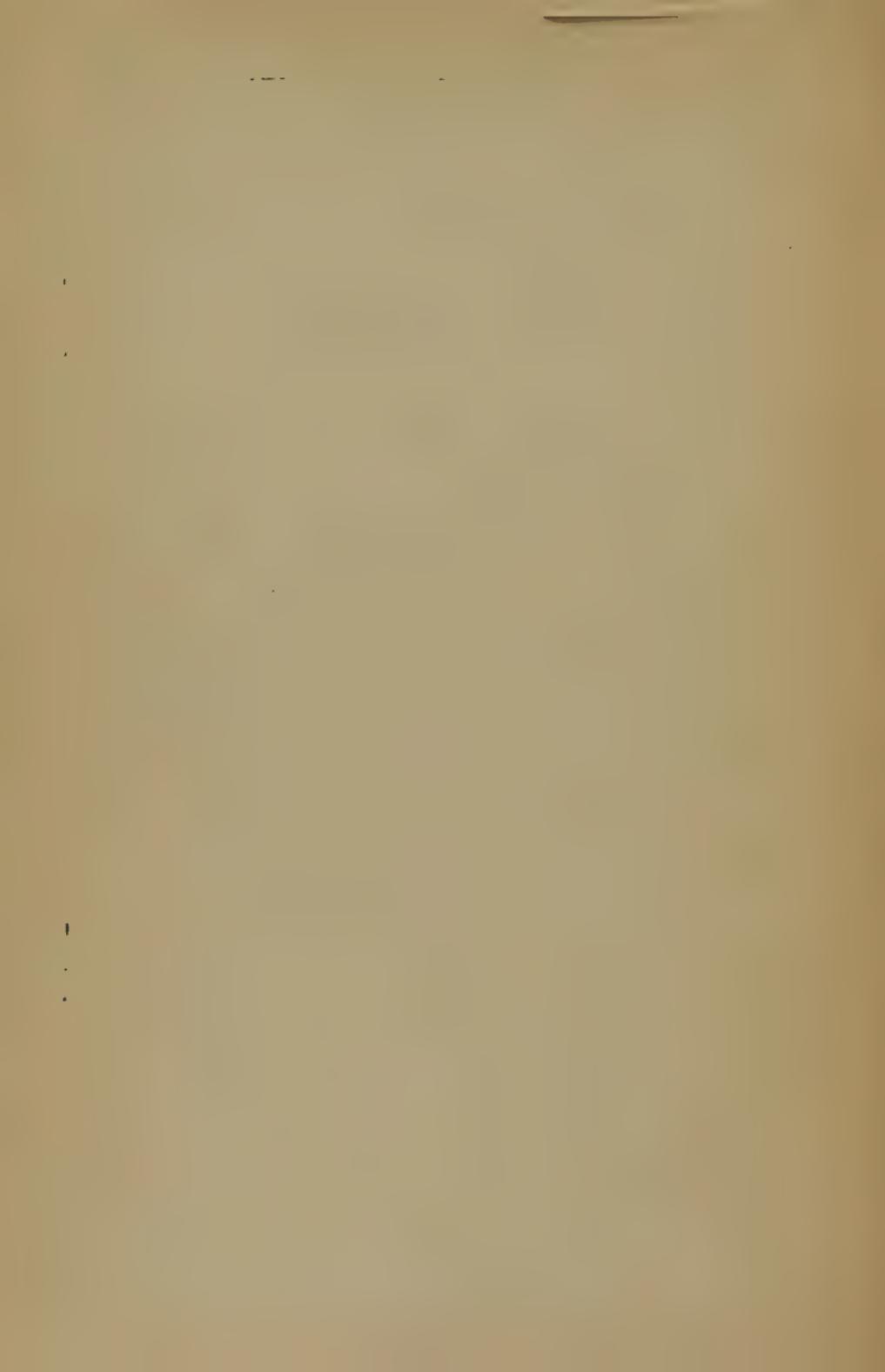
vacuum in the literature of microscopy the present manualette has been prepared. Little claim is made to originality, yet the book is by no means a mere compilation, but the outcome of long and extensive personal experience in the cutting and mounting of microscopical sections. Every process described has been put to the test of actual trial, so that its worth may confidently be depended upon. Many of the little points insisted upon in the ensuing pages will doubtless to the practised microscopist appear superfluous or even puerile ; but a vivid recollection of our own early failures and disappointments assures us that it is just these very *minutiae* of detail which will be found most serviceable in directing and sustaining the faltering footsteps of the tyro

ST. HELENS, *September, 1878.*

PREFACE TO AMERICAN EDITION.

DR. MARSH's book cannot fail to promote the practical study of histology wherever the English language is spoken, and under ordinary circumstances the students of this country might have been left to the use of the London edition. But a careful reading showed that one or two points which are perfectly clear to British readers might be a source of doubt and difficulty to students on this side. At the present day in this country histology is a favorite study with very many private students who have no access to teachers or large libraries, and for the purpose of smoothing their path we have added such information as was obviously desirable. In the hope that these additions will prove of value, we submit the book to American readers.

NEW YORK, *January, 1879.*



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SECTION-CUTTING.

PART I.

1. *Introduction.*—Many of the most interesting objects with which the microscopist has to deal, cannot be made to reveal their beauty or minute structure until they have been cut into slices or *sections*, of such a degree of thinness as to render them transparent, and thus permit of their examination by transmitted light, with objectives of varying power. Unfortunately, however, very few of the objects of this class are, in their natural condition, in a suitable state to be submitted to this method of procedure. Some are of such a soft and yielding nature that any attempt to cut them is an utter failure, for in place of a perfect section being obtained, nothing remains upon the knife but a mass of diffluent pulp ; others, again, are of such density as to resist the action of any cutting instrument.

It is evident, therefore, that nothing can be done with such refractory materials until, by subjection to appropriate methods of preparation, they shall have been reduced to such a consistence as to render them suitable for cutting. How this is to be accomplished will depend entirely upon the physical and chemical

nature of the substance to be operated upon. As the various objects differ so widely from each other in these respects, so must the methods of preparation suitable to each also vary. It is clear, therefore, that no general directions for attaining this end can be given which would be of any practical value. It is possible, however, and very convenient, to arrange the various objects into groups or classes, to the treatment of each of which certain general rules are applicable ; but there will still remain a comparatively numerous series of objects whose individual peculiarities of structure will demand for them correspondingly special methods of preparation. When such objects come to be spoken of, the particular treatment most suitable to each will also be noticed.

2. *On Cutting unprepared Vegetable Tissues.*—There are some few substances, however, which may with more or less success be cut into sections whilst in their natural condition. Such objects are to be found in the vegetable world in certain kinds of leaves and allied structures, whilst in the animal kingdom they are principally represented by the various internal organs of man and the lower animals. Special directions are given in text-books for the preparation of sections of leaves and similar substances. For instance, it is recommended to lay the leaf, etc., on a piece of fine cork, and with a sharp knife to shave off thin slices, cutting down upon the cork. Another plan is to place the leaf, etc., between two thin layers of cork, and cut through the mass. No method, however, is at once so simple and successful as the process of imbedding in

paraffine. To do this, it is necessary to make a paper mould by twisting a strip of stout writing paper round a ruler, and turning-in the paper over the end of the ruler. This mould, the height of which may vary from an inch to an inch and a half, should now be about half filled with melted paraffine mixture (§ 11), the leaf or other object plunged into it, and held in position by small forceps till the paraffine has become sufficiently solidified to yield it a support. More of the paraffine mixture is now poured in until the specimen is thoroughly imbedded ; the whole is to be put away in a cold place for an hour or so, when the mass will be found sufficiently firm to be cut with ease. Sections may be made with a razor kept constantly wetted with water, or, if the preservation of colour be no object, methylated spirit may be employed for the purpose. As the subsequent treatment of such sections in no wise differs from that required by those cut in the microtome, we shall defer its consideration until that method of section has been described (§ 12).

3. *On Cutting unprepared Animal Tissues.*—For the cutting of fresh *animal* tissues several plans may be followed. Thus, if a section of only very limited area be required, it may be obtained by snipping a piece off the tissue with a pair of bent scissors, which, for this purpose, are so made that the blades are *curved on the flat* (Carpenter). If this be carefully performed it will be found that a large portion of the section (particularly at the circumference) so obtained will be sufficiently thin for examination. If a larger section be desired, an attempt may be made to cut it with a

very sharp scalpel or razor, the blade of which whilst in use must be kept *flooded* with water, or spirit, the latter of which is to be preferred. Recourse may also be had to *Valentin's* knife. This consists of two long, narrow blades, running parallel to each other, the distance at which the blades are held apart, and which, of course, determines the thickness of the section, being regulated by means of a fine screw passing through both blades. A milled head attached to this screw gives a ready means of opening or closing the blades, so as to bring them to the desired degree of approximation. The method of using the knife is very simple. After having "set" the blades at the desired distance apart by means of the milled head, the tissue to be cut is held in the left hand immersed in a basin of water. The knife is now steadily and with a rapid motion *drawn* through the tissue, care being taken that the cut is made in such a manner that the blades move from heel to point. By slightly separating the blades and gently shaking them in the water, the section at once becomes disengaged. After use, the blades must be thoroughly dried, when they may be smeared with some oil which does not readily oxidize. For this purpose, a very suitable oil is that known as "Rangoon."* Though it has been deemed advisable briefly to describe the preceding methods of cutting unhardened tissues, it will be found that for the purposes of the ordinary microscopical student sections so obtained are of very little value. They are always of very limited dimensions, seldom of uniform thickness, and often so extremely

* Note A.

friable as to render it very difficult and frequently impossible to submit them with safety to such further treatment as is necessary to fit them for being mounted as permanent objects. This method of section-cutting, however, is not without its uses, for by its means the medical practitioner is provided with a simple and ready method of roughly investigating the structure of morbid tissues, whilst to the general student it furnishes an easy means of making a cursory examination of certain substances, in order that he may determine whether it be worth his while to subject them to some of those various processes of hardening hereafter to be described.

4. *Preparation of Vegetable Tissues.*—Let us now pass to a brief consideration of the methods usually adopted for preparing the various objects for easy section. In the case of *vegetable* tissues, not only do we, as a rule, find their texture of too great density to be readily cut in their natural condition, but they also contain much resinous and starchy matter, of which it is highly desirable to get rid. In order to do this we first cut the substance (say a stem or root) into small pieces, which are to be placed in water for three or four days, by which time all the soluble gummy matters will have disappeared. The pieces are now transferred to a wide-necked bottle, containing methylated spirit, * which, in the course of a few days, will dissolve out all the resin, etc. Many kinds of woody tissue are by these processes reduced to a fit condition for immediate cutting ; others, however, are so hard as to render it

* Note B.

necessary to give them another soaking for some hours in water, to bring them to a sufficient degree of softness to cut easily. If the wood (as in some few refractory cases will happen) be still too hard for section, a short immersion in warm, or if necessary, in boiling water, will not fail effectually to soften it. The treatment of such members of the vegetable division as require peculiar methods, will be found described in future pages.

5. *Preparation of Animal Tissues.*—*Animal* tissues differ from one another so greatly, both in consistence and in chemical composition, as well as in their degree of natural hardness, that no general rules can be given which would be applicable to the preparation of the whole class. Such as are of any considerable degree of hardness, as horn and kindred structures, must be treated much in the same manner as the denser varieties of wood, viz., by more or less prolonged immersion in water—cold, hot, or boiling. Those which are of extreme hardness, as bones and teeth, can be cut only by following certain special methods, full details of which will be found in the Second Part of this work (§26). Many, and indeed the vast majority of animal tissues, offer a direct contrast in point of hardness to those we have just been considering. All the internal organs of the body are, when freshly removed, of much too soft a nature to permit, when in their unprepared condition, of easy or perfect cutting. It is upon bringing them to that critical degree of hardness, which is often so difficult to attain, that the chief secret of successful section-cutting depends ; for unless

the hardening process has been carried up to, but not beyond, a given point, which varies with different tissues, the operator, however dexterous, will fail to obtain satisfactory sections. For, if the hardening has fallen short of this critical point, he is, to some extent, in the same position as if he were dealing with unhardened tissues ; whilst, if this point has been exceeded, the tissue will have become so brittle as to crumble before the knife. For the purpose of hardening animal tissues, the student has at his command two principal agents, namely, alcohol and chromic acid, each of which possesses advantages of its own, but the use of each of which is also attended by its own inconveniences. Thus, by the use of alcohol, there is very much less risk of overhardening the specimen than if chromic acid had been employed. Alcohol, however, though a capital indurating agent in some instances, does not answer so well in many others. Chromic acid is, therefore, to be preferred for general use. It is, however, a very delicate agent to manage, for unless the greatest care be taken it is exceedingly likely to overharden tissues submitted to its action, and when this happens the specimen becomes utterly useless for cutting, as there is no known means of removing the extreme brittleness which it has acquired. By taking the precautions now to be given, this overhardening may generally be avoided. Let us harden a portion of some viscus, say the kidney, for instance. Suppose we cut from the organ five or six small pieces (from half to three-quarters of an inch square, *not larger*). These must be placed in a mixture of equal parts of methy-

lated spirit and water for three days, at the end of which period they may be transferred to a solution of chromic acid, made by dissolving twenty grains of the pure acid in sixteen ounces of distilled water. The solution should be kept in a wide-necked bottle furnished with a glass stopper. At the expiration of seven days, pour off the solution and replace it by fresh. At the end of another week, carefully examine the immersed tissues, and by means of a sharp razor see if they have acquired the necessary degree of hardness to allow of a section of *moderate* thinness being made. If so, remove the pieces and put them into a stoppered bottle containing from six to eight ounces of methylated spirit. If, however, the hardening be found not to be sufficiently advanced, the chromic acid solution is to be poured off and again replaced by fresh. It will now be necessary to examine the tissues at intervals of about two days, until they are found to be sufficiently hard, when they must be transferred to the spirit. Under no circumstances, however, should they be permitted to remain in the chromic acid longer than the end of the third week, and though they should at this time appear not to have undergone sufficient induration, yet it will be advisable to transfer them to the methylated spirit, which in a short time will *safely* complete the process of hardening, without any risk being run of the tissue becoming ruinously brittle. It will be noticed, that when the specimens have been transferred to spirit, the latter will in a day or two become of a deep yellow color, whilst a thick flocculent deposit falls to the bottom of the bottle. The tissues

should then be removed, the bottle emptied and well washed, and, being refilled with clean spirit, the preparations are again to be replaced. This may occasionally be repeated, until the spirit becomes and remains perfectly bright and clear. The specimens are then ready for section.

6. *Special Methods of Hardening.*—The brain (§ 27), spinal cord (§ 43), liver (§ 34), and several other organs, etc., require special methods of hardening, details of which will be found in the paragraph devoted to each. In the case of *injected* preparations, the best plan is to harden them in alcohol from the outset, beginning with weak spirit, and gradually increasing the strength as the hardening proceeds. When the object has been injected with Prussian blue, a few drops of hydrochloric acid should be added to the alcohol to fix the color.

It may here be observed, that specimens of *morbid tissues* require, as a general rule, a shorter immersion in chromic acid solution than healthy tissues do. A very small degree of overhardening speedily renders them brittle and useless. They should, therefore, be removed from the acid medium at the end of ten days or a fortnight, and their further hardening carried on by means of alcohol.

7. *Cutting Hardened Tissues by Hand.*—Our material being now reduced to a fit condition for cutting, let us proceed to consider the several methods by which this may be effected. The readiest and most simple plan, if the piece be large enough, is to hold it in the left hand, and, having brought the surface to a perfect

level by cutting off several rather thick slices, endeavor to cut a thin section by the aid of a very sharp razor, the blade of which must be kept well *flooded* with spirit. As in the use of *Valentin's* knife, so here, great care must be taken steadily to *draw* the blade across the tissue, every effort being made to avoid *pushing* the knife, else the section will be *torn off*, instead of being *cut*. Though this method* is of very great importance for many purposes, yet a considerable degree of manipulative skill is required to enable the operator to obtain anything like perfect sections by its means, and, unfortunately, this skill is acquired by very few persons indeed, even after much practice. If the piece which it is desired to cut be too small to be conveniently held in the hand, it may be imbedded in paraffine in the manner already described (§ 3). A very simple imbedding agent, and one of the greatest practical value, is a strong solution of gum arabic, which, upon being dehydrated either by ordinary drying or the action of alcohol, soon acquires such a degree of hardness as to permit it (with the imbedded tissue) to be cut with ease. As this method of imbedding, however, is most frequently resorted to where, by its means, special difficulties have to be overcome, a full description of the process (§ 35) will be deferred until such special cases come to be spoken of.

8. *Microtome*.—Although the preceding plans may be sufficient to answer all his requirements, if the student wishes to obtain only one or two sections of small dimensions, of a given object, if he requires a number

* Note C.

of such sections he will find these methods fail him, for even though by practice he may have attained to considerable aptitude in the use of the knife, it will still unquestionably happen that the vast majority of his sections will be more or less imperfect. If, therefore, it be desired to procure a number of perfect sections, of equable thickness and large area, it is absolutely necessary to resort to the use of some form or other of microtome, or section-cutter. This instrument, in its simplest form, merely consists of a stout brass tube closed at one end, and being by the other fixed at right angles into a smooth plate of metal. A plug or disk of brass, accurately fitting the interior of the tube, is acted upon by a fine threaded screw piercing the base of the tube, and by means of which the plug, and any object it may support, can be elevated at pleasure. The object by this means being made gradually to rise out of the tube, sections are cut from it by simply gliding a sharp knife along the smooth cutting plate, and hence across the specimen. Any intelligent worker in brass would make an instrument of this kind at a very small cost, and although perhaps it might lack the finish of an instrument bought at the optician's, it would, if accurately made, do its work as well as the most complicated and expensive. If, however, the student resolves to purchase a microtome, there are a variety of forms in the market from which he may choose. A few hints may perhaps be of service in enabling him to make a judicious selection. At the outset we may say that unless the student intends to devote himself solely to the production of sections of wood, etc., he ought not to pro-

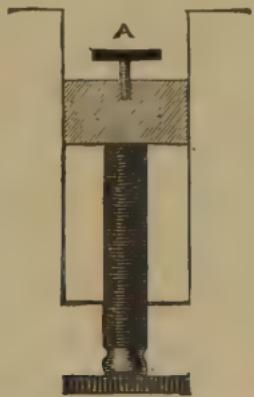
cure one of those forms of microtome known as wood section-cutters, in which the object to be cut is held in position in the tube by means of a binding screw which pierces its side.* Although these machines are all that can be desired for cutting hard bodies, they are not so suitable for soft ones. The chief points to be attended to in selecting a microtome are, (1) that the cutting-plate of the instrument be made of glass, or in default of this, of very hard metal of the most perfect smoothness ; † (2) that the diameter of the tube be neither too large nor too small—it ought not to be less than $\frac{1}{8}$ —in., or greater than 1 inch; ‡ (3) that the screw, which should be *fine* and well cut, be provided with a graduated head ; (4) that there be some kind of index by which fractional portions of a revolution of the screw may be measured; and (5) that the plug fit the tube of the microtome so accurately that when melted paraffine, gum, or other imbedding agent be poured into it, it may not find its way between the plug and side of the tube (§ 18). It often happens in cutting tissues imbedded in paraffine, that the pressure of the knife causes the cylinder of the imbedding agent to twist round in the tube of the machine, and so cause considerable difficulty and annoyance. This evil is usually met by running a deep groove across the upper surface of the plug, and into this the paraffine sinks, and so is prevented from rotating. It will be found, moreover, that another difficulty of a kindred, though much more serious character, will frequently be encountered. During section the paraffine has a tendency not only to

* Note D.

† Note E.

‡ Note F.

rotate, but also to become loosened from the subjacent plug, and to *rise* in the tube of the microtome. When this happens the power to cut sections of uniform thickness has completely gone, for some will now be found to be many times thicker than others; in fact, the irregularity in this respect soon becomes so monstrous as to render it useless to prolong the sitting. In the ordinary run of microtomes no provision seems to have been made to meet this difficulty, and for this reason many instruments, of otherwise great merit, have their efficiency seriously impaired. Fortunately, this imperfection is easily remedied, all that is required being that the upper surface of the plug should be furnished with some kind of projection, having at its summit a table-like expansion, as shown at A in the figure. The imbedding paraffine, by penetrating beneath and



SECTION OF MICROTOME-TUBE SHOWING ARRANGEMENT (A) TO PREVENT
"RISE" OF PARAFFINE.

around this, becomes firmly attached to the plug, and thus all risk of its rising is effectually avoided. If the student wishes to secure a really first-class instrument,

none can be so confidently recommended as the freezing microtome of Professor Rutherford. In addition to its being the best instrument for carrying out the freezing method (§ 18), this machine is equally effective for cutting tissues imbedded in paraffine, or any of the other agents used for that purpose ; indeed, whatever work a microtome *can* do, *this one* will perform.

9. *Aether Microtome*.—A word here as to freezing microtomes, where the agent used is æther. Such as have fallen under our notice have not answered the expectations we were justly entitled to form of them. That it is possible to freeze a piece of tissue by their use is undeniable, but it is, as a rule, at an expenditure of such a quantity of æther (only the very best of which must be used) as to constitute it a very expensive proceeding. Another serious disadvantage they possess is, that if the supply of æther be intermittent for only a very short time, the already frozen tissue thaws with great and most inconvenient rapidity.

10. *Section-Knife*.—Of not less importance than the microtome is the section-knife, to be used in conjunction with it. How perfect soever the former, and whatever the dexterity of the operator, unless he be provided with a suitable and well-made knife, he will never succeed in obtaining satisfactory results. As to the most desirable *size* of the knife, much difference of opinion seems to exist, section knives varying in this respect from a blade of extreme shortness to one which fell under our observation, in which the portentous length of *thirteen* inches was attained. What advantages were to be expected by prolonging the blade to

this extravagant length, must remain an inscrutable mystery to all save its designer. Concerning the *shape* of the knife, it is frequently advised that the surface which has to glide along the cutting-plate of the microtome should be ground *flat*. A most unsuitable arrangement, as a very little actual experience of section-cutting will speedily demonstrate. After many unsuccessful attempts to obtain a really good and reliable section-knife, we determined to have one specially made, which, as it has proved everything that could be desired, merits a brief description. It is of the utmost importance that the blade be made of good and well-tempered steel, not only that it may be capable of receiving an edge of the most exquisite keenness, but also that it may *retain it*. The knife of which we speak (and which was made by Mr. Gardner, of South Bridge, Edinburgh) is furnished with a blade *four inches* long, and $\frac{1}{8}$ -inch broad, set into a square handle of boxwood, also four inches in length. The thickness of the blade at the back is not quite $\frac{1}{4}$ -inch, while *both* of its surfaces are slightly hollow ground. It is essentially necessary that the back and edge of the blade be strictly parallel to each other, otherwise the knife, when in use, will have such a tendency to tilt over as to render its management extremely difficult. It is very easy to discover if this condition be fulfilled, for if on carefully laying the flat of the blade upon a piece of level glass, every portion of both back and edge are found to be in close contact with it, the knife may in this respect be considered perfect. Every student who aspires to be a successful section-cutter should provide

himself with a good Turkey oilstone, *and learn to use it.* He should also possess a razor strop, as it will be in constant requisition.* It may here be remarked that though *razors*, as a rule, are unsuitable for use with the microtome from want of uniformity in the thickness of their blades, yet, if only a small object is to be cut—for instance, a thin root or stem—very good results may be obtained from their use, especially if one of the old-fashioned make, having a thick back and slightly *concave* surfaces, be employed.

11. *Imbedding in Paraffine for Microtome.*—Having described at some length the various instruments necessary for section-cutting, we will now consider how they are to be used. Let us endeavor to cut some sections—say of a piece of kidney—and in so doing we will adopt the “paraffine” method of imbedding. Ordinary paraffine, however, when used alone, is rather too hard for our purpose. In order, therefore, to bring it to a suitable consistence, it must be mixed with one-fifth its weight of common unsalted lard, a gentle heat applied, and the two thoroughly stirred together. A quantity of this should be prepared, so that it may always be ready when wanted—it is very conveniently kept in an ointment pot or preserve jar, the top of the latter being well covered, to keep out the dust. When it is intended to use this mixture for the purpose of imbedding, only just about the quantity required should be melted; for in doing this it is advisable to use as low a degree of heat as possible, not only to prevent injury to the tissue to be imbedded,

* See Note G.

but also that the paraffine when cooling may not undergo such an amount of contraction as to cause it to shrink from the sides of the microtome tube. It is therefore a good plan to effect the melting in a water-bath, a simple kind of which, something after the fashion of a *glue-pot*, would be made for a few pence by any tinman.

The kidney which we are about to cut has, of course, gone through the process of hardening already described (§ 5), and is now preserved in spirit. A small piece, say half an inch square, is selected, removed with forceps, and placed on a bit of blotting paper, when the surface of the tissue will rapidly become dry (*only the surface* must be allowed to dry). It is the usual plan now to proceed at once to imbed it in the melted paraffine. This is a most undesirable step, and gives rise at a later stage of our proceedings to a great amount of trouble and annoyance, for after sections have been cut from a tissue so imbedded it will be found that portions of paraffine adhere to their edges with such tenacity that in the case of many of them there is no effectual method of removing the paraffine, short of soaking the sections in warm æther; a very objectionable proceeding, for though the æther will undoubtedly remove the paraffine, it will also dissolve out any fatty matters which the section itself may naturally contain. All this annoyance may be prevented by subjecting the tissue to a simple preparatory treatment before it is imbedded in the paraffine. For this purpose prepare a very *weak* solution of gum arabic in water—twenty grains to the ounce. Into this, by

means of the forceps, dip for a few moments the already *surface-dried* tissue, taking special care not to squeeze it, or the pressure will cause the spirit from its interior to remoisten the surface, which would prevent the gum from adhering. We shall see the value of this a little later on. Remove the tissue from the solution on to blotting paper, when the superfluous gum will speedily drain off, and in two or three minutes the *surface* will have become quite glazy and dry. Having melted some paraffine mixture in the water-bath, the tissue held in the forceps must be plunged for an instant into the heated liquid and immediately withdrawn, when the crust of paraffine with which it is enveloped will promptly harden. Whilst this is taking place we may make ready the microtome. Having by means of the milled head or handle depressed the plug in the tube so as to leave a free opening about an inch deep at its upper end, we must pour in the melted paraffine, which by this time will have become a little cooler, until the cavity be about half filled. The prepared tissue must now be introduced, care being taken to place it in such a position that the sections may be cut in the desired direction. The tissue must, if necessary, be held in position with forceps or a needle point, till the imbedding material becomes hard enough to give it due support. It is here to be remembered that it will not be advisable to place the tissue in the centre of the tube—it will be much more easily cut if placed rather nearer to that edge of the tube which is situated next the operator in the act of cutting. More paraffine is to be slowly added, until the tissue is com-

pletely covered ; even after this still more should be added, for it will be found that in cooling the paraffine shrinks so as to leave a cup-shaped depression in its centre, whereby portions of the tissue which were previously covered are again laid bare. The best method of preventing this is to use the paraffine at as low a temperature as possible, and to use plenty of it. The microtome, with its contents, must now be removed to a cool place, when the paraffine will soon become solidified. Whilst this is being accomplished we may make our further preparations. The first thing we require will be a large basin, full of freshly filtered water, and provided with a cover. A small beaker of methylated spirit, with a dipping rod or pipette, will also be necessary. We must now see that the section-knife is in thorough order, to ensure which it will be advisable to give it a few turns on the strop. An ordinary razor will also be of service.

12. *Employment of Microtome.*—The paraffine being sufficiently hard, we will clamp the microtome on to the table, and seat ourselves on a chair of convenient height before it. To our right stand the basin of water, razor, and section-knife ; the beaker of spirit to the left, and a cloth on our knee. A few turns of the microtome screw having brought the paraffine to the surface, a thick slice is to be cut off, and this repeated until the imbedded tissue comes into view. This preliminary work had best be done with the razor, as it is needless to subject our section-knife to unnecessary wear and tear. By a fractional revolution of the screw the tissue is now slightly elevated, and with the pipette

held in the left hand, a large drop of spirit is to be let fall upon its surface. The section-knife, grasped firmly

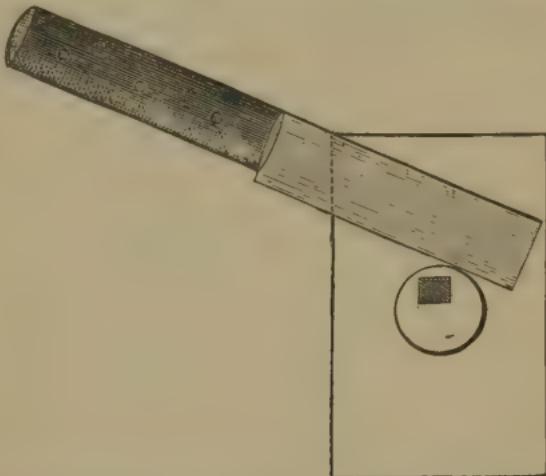


DIAGRAM SHOWING DIAGONAL POSITION OF KNIFE IN COMMENCING TO
MAKE A SECTION.

but lightly in the right hand, is to be laid flat upon the cutting plate of the machine, so as to occupy the diagonal position shown in the figure. Two fingers of the left hand are now laid gently upon the back of the blade, so as to give it an equable support, whilst the knife with a rapid motion is pushed in the combined direction of *forwards* and to the *left*, so that the blade in cutting the tissue will pass through it from point to heel. Thus it will be observed that the stroke of the knife is *from* the operator—a far easier and more effective mode of cutting than the reverse plan. The blade of the knife, having the section just cut, either floating in a small pool of spirit on its surface or adhering thereto, must now be immersed in the basin of water, when by a little very gentle agitation of the

knife the section will be floated off. And now we shall find the great practical value of immersing the tissue in gum before imbedding, for no sooner is the section disengaged from the knife than the thin film of gum which separates the paraffine from it becomes dissolved, and the section will be observed gradually to subside to the bottom, leaving the paraffine floating upon the surface. After carefully wiping the knife from all shreds of paraffine, the microtome screw must again be partially revolved, more spirit applied to the tissue, and another section being cut, it must be transferred to the water as before, and so on, until a sufficient number of sections have been obtained. As to how thin the sections should be cut, no general directions can be given ; each case must be regulated by its own conditions. The denser the tissue, the thinner should the section be ; whilst certain substances of loose and spongy texture do not require the sections to be particularly thin—it may be said, however, in a general way that sections, and especially animal ones, *cannot be cut too thin* so long as they remain perfect and entire. If Professor Rutherford's microtome (as made by Gardner) be employed, the head of the screw will be found to be graduated into divisions of slightly unequal value ; the sections will therefore be marked by corresponding variations of thickness, so that amongst a number cut, there must be many of the exact thickness to meet the requirements of any individual case.

13. *Staining Agents.*—Before proceeding to mount the sections which have just been cut, it will be very advisable that they should be submitted to the action

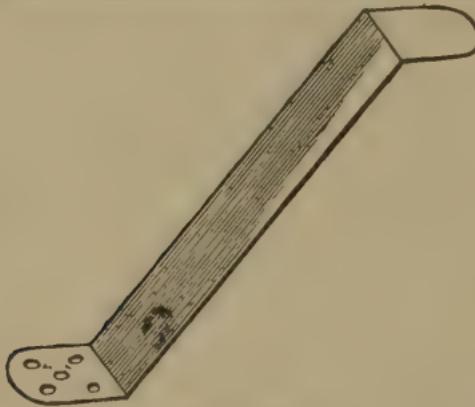
of some staining fluid, in order to render more clear and distinct their minute structure. Organic substances possess the property of being able to absorb various colouring matters from their solution, and to incorporate such colour into their own texture. This power of attraction is not, however, possessed by all substances indiscriminately, or to an equal extent. Some possess it in a high degree, while others appear to be nearly, if not entirely, devoid of such power. Hence it follows, that if we immerse an organic tissue (one of our sections, for instance) of complex structure, in a suitable staining fluid, the tissue will not become stained in an even and uniform manner throughout, but the several portions of it will receive varying depths of colour in accordance with the varying attractive power of its several constituents. By this means we are enabled in stained sections to discriminate by their difference of shade, minute and delicate structures, which in the unstained condition it would be difficult and often impossible to differentiate. For the purpose of section-staining there are many agents in use, the most generally suitable being carmine, logwood (§ 19), and aniline blue (§ 27); whilst for special purposes chloride of gold (§ 28), pyrogallate of iron (§ 28), and several others are all of much value.

14. *Carmine Staining.*—In the case of animal sections, carmine is, as a rule, to be selected, giving as it does most satisfactory and beautiful results. Tissues may be stained with carmine by two different plans: in the first, a strong solution is used, and the tissue subjected to its action for a very short period only, whilst

in the latter only very weak solutions are employed, the time of immersion being considerably prolonged. The rapid method, however, is not to be recommended, for the strong carmine acts so powerfully upon the tissue as to give the various elements comprising it no time, as it were, to exercise their power of quantitative selection, but involves the whole in one uniform degree of shadeless colour. By adopting the gradual method much better results are obtained, each portion of the tissue being now at liberty to acquire its own particular shade. Amongst the various formulæ for the preparation of carmine fluid, none can be so safely followed as that devised by Dr. Lionel Beale. It runs thus:— Place ten grains of the finest carmine in a test tube, add thirty minims of strong liquor ammonia, boil, add two ounces of distilled water, and filter; then add two ounces of glycerine, and half an ounce of rectified spirit—this solution ought to be kept in a well stoppered bottle. The best vessels in which to stain sections are small jars of white porcelain, capable of holding about two fluid ounces, and furnished with lids—they are much preferable to beakers or watch glasses, for owing to the white background which they afford it is very easy to watch how the staining is proceeding. The carmine solution which we have just described is both too strong and of too great density to be used in its pure state. It will, therefore, require to be diluted with distilled water before use—the most useful degree of dilution being attained by adding one part of stain to seven of water. Sections may be placed in this solution for twenty-four hours, in which time they will

usually be found to have acquired a sufficient depth of colour. If, however, the tissue be unusually difficult to stain, the time of immersion may be doubled, or still further prolonged, without detriment to the section.

Having prepared and filtered some of this dilute solution, say an ounce, let us proceed to stain with it those sections which we left in the basin of water (§ 12). Here we are at once met by a practical difficulty. How are the sections to be transferred from one vessel to the other? This is ordinarily effected by means of a soft camel's-hair pencil. It is a method, however, open to grave objections, for the sections so curl around the brush, and get entangled amidst its hairs, that, notwithstanding every care, valuable sections not unfrequently become torn during transit. Every difficulty at once vanishes if we substitute for the brush a small implement, which any one can readily make for him-



SECTION SPOON.

self. All that is necessary is to take a strip of German silver, or copper, of the thickness of stout cardboard, and about seven inches in length by five-eighths of an

inch in breadth. The sharp angles are to be filed off and the edges carefully smoothed, whilst at a distance of five-eighths of an inch from each extremity the end must be turned up so as to form an angle of about 35° . One end must be left plain, whilst the other, with the aid of a punch or drill, is to be pierced with five holes about the thickness of a stocking needle * (see Figure). If we now dip the perforated end of this spoon into the water containing the sections, and gently agitate it, the sections will rise from the bottom and float about. The spoon is now brought under one of them, and being steadily lifted up the water flows downwards through its apertures, and the section smoothly spreading itself out upon the spoon, may be gently lifted out of the water, and on the spoon being dipped into the staining fluid the section at once floats off. By this simple means sections, however large, thin or delicate, may with ease be conveyed from one fluid to another, with the utmost certainty of their not being injured during the process. The sections having been in the carmine fluid for about twenty-four hours, as much of the liquor as is possible must be gently poured off, and its place supplied by a freshly-filtered mixture of five

* Dr. Klein describes a kind of "lifter," made by bending some German-silver wire, but as no drawing accompanies his description, it is not easy to form a clear idea as to the form of this instrument. In the recent and philosophical work of Schäfer, a lifter is figured, which consists of a wire stem, having attached to its end a spade-like blade. It will be observed that the spoon described in the text differs from this lifter in having one end perforated, and in this consists the real value of the implement.

drops of glacial-acetic acid to one ounce of water, when in a few moments the carmine will become permanently *fixed* in the tissue, and the process of staining be complete.

15. *Mounting Media*.—The further treatment of the stained sections will entirely depend upon the nature of the medium in which it is intended to mount them. There are a variety of fluids in use for this purpose, the principal being dilute alcohol (§ 26), dammar, or Canada balsam (§ 22-23), and glycerine. These, however, cannot be used indiscriminately, each possessing certain special properties which render it suitable for use with particular classes of objects only. Thus, weak spirit, having no tendency to increase the transparency of objects, can advantageously be used with such only as are already perfectly transparent. It is also more suitable for the preservation of vegetable tissues (when the retention of colour is no object) than animal, since with the latter it has a tendency after a while to cause a kind of granular disintegration, which ultimately destroys much of the usefulness of the preparation. Dammar and Canada balsam, on the other hand, possess very great refractive power, so that they are of great service in mounting objects which require their transparency to be much increased. For this reason they are not well adapted to the preservation of very delicate or transparent tissues (unless previously stained), the minute details of which become almost entirely obliterated when mounted in them. The chief advantage possessed by these resinous media is, that tissues mounted in them undergo no alteration,

even after the lapse of many years. Glycerine, in respect of its clarifying powers, occupies an intermediate position between spirit and balsam, being much more refractive than the former, infinitely less so than the latter. It is, therefore, of very great value for the preservation of such tissues as possess a medium degree of transparency, and which would become obscured if mounted in spirit, or have their outlines rendered indistinct if preserved in balsam. It is of the utmost value for mounting unstained anatomical sections which, when put up in this medium, reveal such minute details of structure as would readily have escaped observation had any other agent been employed. It may also be used with stained sections, but in this case the sections should be of extreme thinness, otherwise the refractive power of the glycerine will be insufficient to render them thoroughly transparent. The great drawback to the use of glycerine is the extreme difficulty experienced in preventing its escape from beneath the covering glass, for it unfortunately possesses such great penetrating power that no cement hitherto devised can be thoroughly depended upon for withstanding its solvent action for any considerable length of time.* Attention to the instructions presently to be given (§ 16) will, however, reduce this risk of leakage to a minimum. In the use of glycerine Dr. Carpenter's caution must ever be borne in mind, viz., that, as carbonate of lime is in time dissolved by glycerine, this agent ought never to be employed for the preservation of objects containing such salt.

Note H.

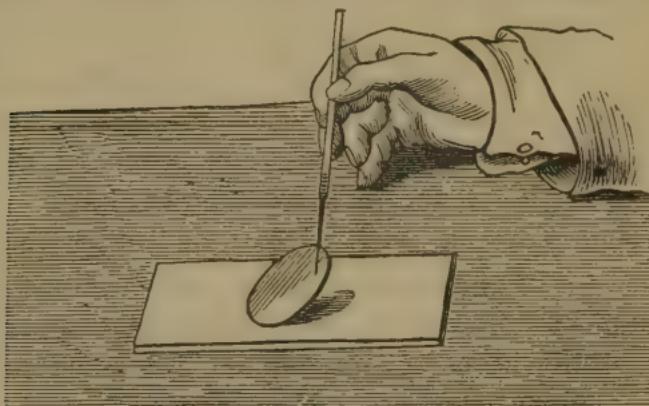
16. *Mounting in Glycerine.*—To illustrate the method of using this medium we will mount our present sections in glycerine. In the first place we shall require a deep watch-glass, which is to be half filled with glycerine diluted with an equal amount of distilled water. By means of the spoon, one or more sections may be transferred into this, either directly from the acetic acid solution (§ 14), or if, since cutting, they have been preserved in spirit, they should first undergo a short immersion in a large vessel full of water. The watch-glass should now be covered with an inverted wine-glass, and put away for some hours, in order that the sections may become thoroughly saturated with the dilute glycerine. When this has been accomplished, a slide must be cleaned, and one of the sections, with the aid of the *unpierced* end of the spoon, be transferred to its centre.* As the kind of section with which we are now dealing is, or ought to be, of ex-

* The appearance of a slide is vastly improved if the preparation be placed *exactly* in its centre. This may readily be done in the following manner:—Take some very finely-powdered Prussian blue, and rub it up in a mortar with a little of the weak gum solution (§ 11), so as to form a thin blue pigment. A quantity of this should be made, so as always to be at hand. A slide having been cleaned, the *best surface* is to be selected, and on the *reverse* side, by means of the self-centring turn-table, a small circle is to be drawn with a camel's-hair pencil, charged with the pigment. In the centre of this ring, but on the opposite side of the slide, the section is to be placed, when it of course will occupy a position exactly central. When the slide comes to be finished, the blue ring may easily be removed with a wet rag.

treme thinness, no cell (§ 26) is necessary. After tilting up one end of the slide, so as to drain off as much of the weak glycerine as possible, a drop of Price's best glycerine must, with a glass rod or pipette, be allowed to fall gently upon the section, so as to avoid the formation of air-bubbles. If any of these, however, should be produced, they must be removed with the point of a needle set in a wooden handle,* and the slide then covered with a small bell-glass (or wine-glass). A circular cover is now to be cleaned with a soft handkerchief, and after gently blowing from it any adhering fibres of lint, etc., it will be advisable to hold the side of the glass which is to come into contact with the preparation close to the mouth, and breathe upon it, so as to cover it with moisture. The cover held between the thumb and forefinger of the left hand must now be applied by its edge near to the margin of the preparation, and the surface of the cover directed in an inclined manner over it. Beneath the overhanging edge of the cover the point of the needle, held in the right hand, is now to be inserted (see Figure). By gently lowering the needle, the cover will come into gradual contact with the slide, driving before it a

* A *crochet-needle* holder made of bone, and which may be bought at the smallware dealers' for about sixpence, makes an admirable handle for microscopical needles. At one extremity there is a small cavity, closed with a cap, for the storage of reserve needles, whilst the other end terminates in a metal tip, provided with a crucial slit and central perforation for the reception of the needle in actual use, and so arranged that, by means of a small screw-nut, needles of various sizes may be firmly held in position.

minute wave of glycerine, in which any air-bubbles that may have become developed are usually carried off.



METHOD OF APPLYING COVER.

A very considerable degree of tact, however, is required to perform this little operation, simple as it may appear, for the retreating wave of glycerine not unfrequently floats out the section, either wholly or partially, from beneath the cover. Air-bubbles, also (*the bêtes noires* of this process), are exceedingly likely to arise. When this happens the best plan to adopt is, by means of the needle point, gently to raise and remove the cover, apply another drop of glycerine to the section, and cover with a *fresh piece of thin glass*. It will now be necessary to remove any superfluous glycerine which may have collected around and near the cover. The great bulk must be wiped away by means of a camel's-hair pencil, slightly wetted between the lips, any remaining stickiness being removed with a bit of blotting paper which has been slightly damped. With a very small camel's-hair pencil, charged with solution of

gelatine, a ring must be made round the margin of the cover, of sufficient breadth to take in a small tract of both cover and slide. As this cement is perfectly miscible with glycerine, it readily unites with any of that fluid which may ooze from beneath the cover, and which, in the case of any of the ordinary varnishes, would act as a fatal obstacle to perfect adhesion. To make the cement, take half an ounce of Nelson's opaque gelatine, put in a small beaker, add sufficient cold water to cover it, and allow the mixture to remain until the gelatine has become thoroughly soaked. The water is now poured off, and heat applied until the gelatine becomes fluid, when three drops of creosote should be well stirred in, and the fluid mixture transferred to a small bottle to solidify. Before use, this compound must be rendered liquid by immersing the bottle containing it in a cup of warm water. When the ring of gelatine has become quite set and dry (which will not take long), every trace of glycerine must be carefully removed from the cover and its neighborhood, by gently swabbing these parts with a large camel's-hair pencil dipped in methylated spirit. After drying the slide a ring of Bell's microscopical cement may be applied over the gelatine, and, when this is dry, another coat is to be laid on. If it be desired to give the slide a neat and tasteful appearance, it is a very easy matter, by means of the turn-table, to lay on a final ring of Brunswick black or white zinc cement (§ 24). Every care has now been taken to render our preparation permanent; but, to make assurance doubly sure, it will be well to follow Dr. Carpenter's advice, and, every

year or so, to lay on a thin coating of good gold size.*

17. *Use of Freezing Microtome.*—Our preceding consideration of the method of employing the microtome in conjunction with paraffine as an imbedding agent (§ 11), will have formed a very suitable introduction to the study of the somewhat more complicated process of imbedding the tissue in gum, for section in the freezing microtome. This method is of the utmost value to the practical histologist, for by its means he is enabled with ease to possess himself of perfect sections of several structures, the cutting of which, before the introduction of this process, was always a matter of difficulty and anxiety. The freezing microtome is especially valuable for the section of such substances as from their extreme delicacy are liable to be injured by being imbedded in paraffine—for instance, the delicate villi of the intestines becomes very frequently, by the use of paraffine, denuded of their epithelium, and the villi themselves not seldom become torn off or otherwise damaged. The great value of the method is also very well seen in the treatment of those tissues which,

* If square covers be employed, they may be fixed to the slide by a simple method much in vogue in Germany. A thin wax taper is to be lighted, and being partially inverted for a few seconds, the wax surrounding the wick will become melted. After the slide has been freed from excess of glycerine, a drop of this heated wax is allowed to fall upon each corner of the cover, and a line of the melted wax run along the margins of the cover between these points, so as perfectly to surround it. If a good coat of white zinc cement be subsequently laid over the wax a very durable, and not unornamental, line of union will have been formed.

like the lung, are of such loose and spongy texture as to offer insufficient resistance to the knife unless their interstices have previously been filled up with some solid yet easily cut material. As the space at our command is strictly limited, we are precluded from entering as fully into this branch of section-cutting as the importance of the subject demands and our own inclination would lead us. To those who wish to become thoroughly conversant with the full value of this method we cannot do better than recommend the perusal of Professor Rutherford's *Practical Histology*, 2d edition, than which, on the whole subject of physiological microscopy, no treatise with which we are acquainted is at once so plain, practical, and profound.

18. *Employment of Freezing Microtome.*—A very suitable object with which to demonstrate the method of using this form of microtome will be afforded us by a portion of intestine, say of the ileum of a cat or dog. Suppose we have some of this in methylated spirit—let us select a piece of about half an inch in length. Our first care will be to deprive this of its spirit; for so long as the tissue remains impregnated with alcohol it would, of course, be impossible to freeze it. We will, therefore, throw it into a large basinful of water, and leave it there for twenty-four hours, during which time it would be as well to change the water once or twice. We shall now require a strong solution of gum. This, which should have been made some time previously, may be prepared by placing a quantity, say three or four ounces, of ordinary gum arabic in a glass beaker, and adding sufficient water to cover it—the mixture

must be stirred occasionally with a glass rod until solution has taken place, which will be in a few days. If necessary a little more water may be added, but so long as the gum will pour from vessel to vessel, it cannot well be made too strong. Mucilage, by keeping, is very apt to become sour and mouldy—this may be prevented, by adding to each ounce of the water with which it is prepared about half a grain of salicylic acid. We now pour some of this mucilage into a small vessel—an egg cup will answer very well—and into it transfer the piece of ileum from the water. Here we must allow it to remain for a time sufficient to permit of its becoming thoroughly saturated with the gum, for which purpose some hours will be necessary. When this soaking has been accomplished we will prepare the microtome, which we will assume to be Rutherford's. In the first place it will be necessary to remove the plug—which is to be done by turning the handle connected with the screw until the plug rises so high in its tube that it may be grasped with the fingers and removed, when it is to be well smeared all over with sperm oil and replaced. This is done to prevent any unpleasant adhesions taking place whilst the freezing is going on. We must next depress the plug, so as to convert the upper part of the tube into a kind of “well” of sufficient depth to hold our specimen. It will now be very advisable to look carefully into this *well* and observe whether the plug fits accurately into the tube (§ 8) for if there be any interval between the two it will give rise to much subsequent annoyance, as the gum penetrating this interstice will there become firmly frozen into

irregular patches, which will so interfere with the even gliding of the plug within its tube as to cause the former to ascend in such an irregular and jerky manner as to be utterly destructive of all accuracy in the cutting. If this defect be observed, it may be at once remedied by dropping a small quantity of gently heated paraffine into the *well*, which will effectually close up any fissures. The microtome, by means of its clamping arrangement, must now be firmly attached to the table, and a suitable vessel be placed on the floor beneath it, so that it may catch the water which will issue from the waste-pipe of the apparatus. The next requirement is a supply of block ice and finely powdered salt. A lump of the ice must be wrapped in a towel, and crushed into small pieces ; these, by means of a large mortar, are to be further reduced to a very *fine powder*. Any attempt to hurry over this troublesome part of the operation will lead to future disappointment, for unless the ice be used in a very fine powder great delay (at least) in the freezing will be the result. With the aid of a small spoon the ice and salt are in alternate spoonfuls to be conveyed into the freezing-box of the machine, great care being taken that the cavity under the cutting-plate and around the tube be thoroughly packed, after which the uncovered portion of the box should also be well filled. The *well* is now to be filled with the strong gum to within a little distance of its top, and a piece of sheet gutta-percha (such as shoe soles are made of) being applied over the well, and kept in position by a weight, we must wait until the freezing commences. In a short time we shall

notice that the gum has acquired a thick muddy appearance. The tissue must now, by means of the forceps, be transferred to the well, and there placed in such a position that the sections, when cut, shall run in the desired direction. After more gum has, if necessary, been added, so as completely to cover the tissue, the well is again to be covered, and attention given to the freezing-box. As the mixture which this contains becomes melted, it must constantly be renewed, care being at the same time taken that the mouth of the discharge-pipe be kept quite free, otherwise water accumulating in the box, the freezing mixture will degenerate into a useless puddle. When the gum becomes sufficiently hard to cut, this must be done much in the same manner as if paraffine had been used (§ 12). In this case, however, no fluid will be required, or must be used, to wet the knife with, and especial care must be taken that in disengaging the sections from the knife into the water they be not torn. These sections often adhere very tenaciously to the blade, but if a little patience be exercised the water will soon float them off in safety—much more safely than if any attempt be made to liberate them prematurely. There is one circumstance connected with the use of the freezing microtome which is rather annoying. The moisture of the breath and atmosphere is apt to become condensed on the cutting-plate, and here, mixed with accidental smears of gum, it becomes frozen into a jagged and irregular sheet of ice, which not only seriously interferes with the smooth play of the knife, but also constitutes a real peril to its edge. As this evil cannot

be avoided, all we can do is, by constant wiping, to keep the cutting-plate clean and free from this accumulation. This is best done with a bit of soft rag *just moistened* with spirit, but this must not come into contact with any portion of the cylinder of frozen gum, else it will instantly thaw it. When using the freezing microtome it is always advisable to wear an apron, otherwise our clothes may receive considerable damage from the constant splashing of the salt water, as it falls from the waste-pipe into the vessel beneath it. After use, the microtome must be well washed in plenty of cold water till every trace of salt be removed, for if any of this remain it will quickly corrode the brass-work of the instrument. The plug and screw, as also the section-knife, should be well smeared with Rangoon oil before the machine is put away.

19. *Logwood Staining.*—The employment of logwood as a staining agent is now becoming very general. It acts much in the same manner as carmine, but the violet color which it produces is by many thought to be of a more soft and agreeable character than that due to the action of carmine. A valuable and very convenient property also which it possesses is that it stains tissues very rapidly, and this without interfering with that differential kind of coloration (§ 14) upon which the chief value of all staining processes depends. A simple method of preparing the logwood fluid is to mix an aqueous solution of extract of logwood with a solution of alum (1 to 8) till the deep impure red colour has become violet, and then to filter the mixture (Frey). This will stain sections in about half an hour. This

stain, though here mentioned for the ease with which it may be made is, as a rule, very inferior to a fluid prepared directly from hæmatoxylon, the alkaloid or active principle of logwood. As, however, it is difficult and troublesome to make the solution in this manner,* it will be advisable for the student to purchase, ready prepared, such small quantity of the dye as he may require. Small bottles may be obtained for a few pence of Mr. Martindale, 10, New Cavendish Street, London, and from repeated trials of this solution we can recommend it as producing excellent results. It is a very strong fluid, and requires to be diluted before use. The degree to which the dilution must be carried cannot, however, be very accurately indicated, for all staining fluids of this nature possess the very undesirable property of becoming decomposed by age. After the fluid has been kept for some time, a portion of the colouring matter is thrown out of solution, and becomes deposited upon the sides and bottom of the vessel in which it is contained, hence the older the preparation, the weaker it will have become. As the time required for staining with logwood is but short, it is desirable that all the sections should begin to be submitted to its action at the same time, otherwise some will become more deeply stained than others. A good plan is to fill a small porcelain jar (§ 14) with filtered water, and into this transfer the sections. Whilst they are settling well down to the bottom, a

* Should the student, however, determine to prepare this solution for himself, he will find a good formula for the purpose in Schäfer's "Practical Histology," p 176. Note I.

mixture must be prepared of half a drachm of Martin-dale's solution (fresh) to one ounce of distilled water, and everything got in readiness for its immediate filtration. The water is now very gently to be poured off the sections, and if care be exercised this may be done in such a manner as to leave them undisturbed at the bottom, after removing almost every drop of water. The diluted logwood fluid must now be *immediately* filtered upon the sections, so that they may run no risk of becoming dry. In the present instance the staining may be allowed to proceed for about thirty minutes, and this will be found a convenient time for the immersion of the general run of animal sections. If the logwood fluid be not quite fresh, either a little more of it will have to be added to the water, or the time of immersion must be prolonged until the desired depth of color has been produced. It is well whilst the staining is going on gently to shake the vessel occasionally, so that the sections may not remain in a heap at the bottom, but all be as fully as possible exposed to the action of the dye. When the staining is judged to be complete, the logwood solution must be gently poured off, leaving the stained sections at the bottom of the jar, when they should be quickly covered with methylated spirit, which will *fix* the colour. We shall now be able to see if the coloration obtained be perfectly satisfactory. If not deep enough, it is very easy again to submit them to the action of the dye for a few minutes longer. If on the other hand, and as more frequently happens, the coloration should be too deep, the excess of colour may readily be removed by transfer-

ring the sections for a short time into some diluted acetic acid prepared by adding five drops of the glacial acid to an ounce of water. The action of this should be carefully watched, and when the colour has been reduced to the desired tint the sections may be retransferred to the methylated spirit.

20. *Absolute Alcohol.*—As we purpose mounting the sections which have just been stained, in Canada balsam, we will briefly consider the preliminary treatment to which they must be submitted before this can be effected. The object of this is to abstract from the tissue all its water, for if any moisture be permitted to remain in the section it will, when mounted in balsam, become obscured and surrounded by a kind of opalescent halo, due to the imperfect penetration of the balsam into the only partially dehydrated tissue. The old-fashioned plan of dehydration was simple exposure to the air. The method now generally adopted is to bring about the same result by means of absolute alcohol. This fluid has such a strong affinity for water that tissues submitted to its influence are rapidly and effectually deprived of any water they may contain. Absolute alcohol in small quantity may be obtained from the druggist at about sixpence per ounce. It will be necessary for the student to provide himself with a little of this agent, say about two ounces, the method of using which will very shortly be explained. Absolute alcohol must be kept in a bottle with a very accurately-fitting stopper, in order to prevent its absorbing moisture from the air. For our purpose such a bottle, having a neck *as wide as possible*, is to be selected.

21. *Clove Oil*.—After being thoroughly dehydrated the sections may, in special instances (§ 48), be at once mounted in balsam ; but, as a general rule, it will be found necessary (particularly in the case of animal sections) to treat them with some clarifying agent, in order to remove the cloudiness and opacity which is (in part) due to their previous immersion in alcohol. For this purpose turpentine, or any of the essential oils, may be used : of these, oil of cloves is to be specially recommended. It is rather expensive, ranging from sixpence to one shilling per ounce : but, as a drop or two will be sufficient for preparing each slide, only a small quantity—say half an ounce or an ounce—need be procured. The most convenient vessel in which to keep the oil is one of the small test bottles used by watchmakers. These bottles are provided with a glass cap to exclude dust, and the stopper is prolonged into a glass rod, which dips into the bottle. The use of this rod and the method of employing the oil will be explained shortly.

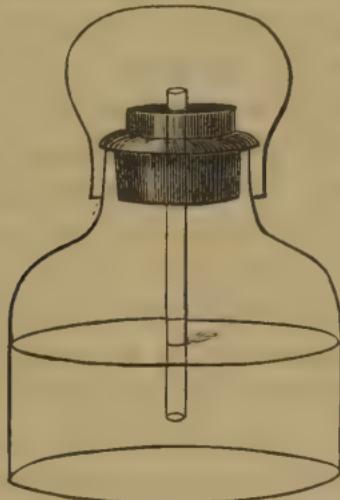
22. *Canada Balsam*, as ordinarily met with, is a thick resinous balm of great viscosity, but readily rendered perfectly fluid by the application of heat. Formerly, sections were mounted in this medium in its pure state, but owing to the annoyance which was so constantly being experienced from the tenacity with which intruding air-bubbles were held by the viscous medium, this plan of mounting is rapidly falling out of use.* It is now usual to employ the balsam in a di-

* Although we cannot too strongly insist upon the use of chloroform-balsam wherever practicable, yet it sometimes hap-

luted condition, the two chief diluents being chloroform and benzole. As balsam, however, often contains more or less moisture, it is desirable to drive this off before adding the diluent. A very convenient way of doing so is to expose some pure balsam to the heat of a cool oven for several hours, when the balsam will be found to have assumed a hard, vitreous character. It should now be broken into small pieces, these put into a bottle, and some methylated chloroform added, which in a little while will completely dissolve the hardened balsam. More chloroform is then to be added, until a

pens in the mounting of substances of *considerable thickness*, that after all the chloroform has evaporated an insufficient amount of balsam is left behind to fill up the cavity between slide and cover. In such cases, therefore, it is advisable to use pure balsam, which may be done in the following manner. The object having been previously thoroughly dehydrated by immersion in absolute alcohol, is to be thence transferred to a little good turpentine, or benzole, where it should remain until perfectly transparent. It is now to be placed in the centre of a slide which has been gently warmed, and a drop or two of *fresh* fluid balsam added, the greatest care being taken to prevent the formation of air-bubbles. Should such arise they must be touched with the point of a heated needle, which will cause them to burst and disappear. The chief difficulty of the process has yet to be encountered in the application of the cover; for it is during this procedure that the development of air-bubbles is most likely to take place. This annoyance may, however, be entirely avoided by taking the simple precaution of dipping the cover into turpentine before it is applied (§ 16), when it will be found that "you can't get air-bubbles, even if you try." The courtesy of Mr. J. A. Kay, of Chatham, enables us to give our readers the benefit of this practical "*wrinkle*."

solution is obtained sufficiently thin to run through filtering-paper. A glass spirit lamp must now be procured, having a capacity of about two ounces, and



SPIRIT LAMP ADAPTED TO CONTAIN BALSAM.

provided with a cap. Into the wick-holder of this (which must be made of porcelain) a hollow glass tube is to be so fitted that its end dips into the lamp to within about a third of the bottom. (See Fig.) The thin chloroform balsam is now to be filtered into this lamp, very fine filtering-paper—through which a little chloroform has first been passed—being used for the purpose. When the lamp is full it must (deprived of its cap) be put in a warm place until sufficient of the chloroform has evaporated to leave behind it a fluid of the consistence of thin syrup.

23. *Mounting in Balsam.*—Let us now return to our sections which, it will be remembered, were left in methylated spirit (§ 19). These we will now mount in

balsam, and although, of course, any number may be proceeded with at the same time, yet to avoid confusion, in the following directions one section only will be spoken of. This section then is, with the perforated spoon, to be transferred to the bottle of absolute alcohol, where it may remain for about an hour—considerably less time is *actually* required, but as from constant use the spirit becomes weakened, it is as well to be on the safe side. It must now be removed to the centre of a clean glass slip, and here the *plain* end of the spoon comes into use. If this be employed for effecting the transfer, it will be found that when the section is being removed from the alcohol it will bring along with it a small pool of the spirit. A slight touch of the needle applied to the edge of the section will cause it to float from the spoon on to the slide, at the same time carrying the pool of alcohol with it, in which it will gently spread itself out upon the slide without the faintest risk of injury. The superfluous spirit is now to be drained off, and just as the section is becoming glazed and sodden-looking (*not dry*) we must, by means of the long glass stopper (§ 21), apply to it a large drop of clove oil. The oil, however, should not be placed *on* the section, but be allowed to drop on to the slide near to its margin. By gently tilting the slide the oil will gradually insinuate itself *beneath* the section and slowly ascend through it to the surface. The slide should now be covered with a bell-glass (or wine-glass), and about two minutes allowed for the oil thoroughly to saturate the section. As much as possible of the superfluous oil must then be drained off, and the re-

mainder removed with blotting paper. By means of the glass rod a small quantity of chloroform balsam is now taken from the spirit lamp which contains it, and allowed gently to fall upon the section, which must then be covered with a thin glass circle in the manner previously described (§ 16). When the object is very fragile, it is a good plan, after draining off the clove oil, to apply the cover directly upon the section, and then to place a drop of the balsam near to the edge of the cover. This, by capillary attraction, will speedily diffuse itself beneath the cover, flowing over and surrounding the object, without in the slightest degree disturbing its position. If, during the process of mounting, any air-bubbles arise, we may view their development with equanimity, being well assured that as the chloroform evaporates they too will quickly disappear. When the mounting is completed, the slide should be roughly labelled and placed on a warm mantel-piece for a few days to dry.

24. *Finishing the Slide.*—In the course of two or three days it will be advisable to take an old penknife, and after heating the blade in the flame of a spirit lamp, gently to run the point of it round the margin of the cover, so as to remove any excess of balsam which may have oozed from beneath it. In a few days more, any remaining balsam may be carefully scraped away with a cold knife. All remaining traces of balsam are then to be removed from around the cover by means of a rag *just moistened* with methylated spirit, or, what is better, with a mixture of equal parts of spirit and æther, after which the slide is to be thoroughly

washed in cold water. The slide is now in reality finished, but, in order to give it a smart appearance, it is usual, with the assistance of the turn-table, to run a ring of colored varnish round the covering glass. A very useful varnish for the purpose is the *white zinc cement*. To prepare this, dissolve an ounce of gum dammar in an ounce of turpentine by the aid of heat. Take one dram of oxide of zinc and an equal quantity of turpentine ; rub them up together in a mortar, adding the turpentine drop by drop, so as to form a creamy mixture perfectly free from lumps or grit. One fluid ounce of the dammar solution previously made must now gradually be added, the mixture being kept constantly stirred (*Frey*). The cement, when made, should be strained through a piece of fine muslin, previously wetted with turpentine, into a small wide-necked bottle, which, instead of having a cork or stopper, should be covered with a loose metal cap. Instead of a bottle, the varnish may be kept in one of the collapsible tubes used by artists ; but though this plan is highly recommended by many, it is not without its disadvantages. If the varnish becomes thick by keeping, a few drops of turpentine or benzole well stirred in will soon reduce it to a suitable consistence for use.

PART II.

25. *Special Methods.*—Having in the preceding pages entered at some length into the general subject of section-cutting, it remains for us now to consider those special methods of preparation which the peculiarities of certain objects demand. In order to keep the bulk (and consequent price) of this manualette within due bounds, we shall, without further preface, proceed to the description of these methods, in doing which every endeavour will be made to employ such brevity of expression as may be consistent with perfect clearness of meaning. As the most convenient plan, the objects here treated of will be arranged in alphabetical succession.

26. *Bone.*—Both transverse and longitudinal sections should be prepared, the former being the prettier and most interesting. After prolonged maceration in water, all fat, etc., must be removed and the bone dried, when as thin a slice as possible is to be cut off in the desired direction, by means of a very fine saw. If the section so obtained be placed upon a piece of smooth cork it may, with the aid of a fine file and the exercise of care, be further reduced in thickness. It is then to be laid upon a hone moistened with water, and being pressed gently and *evenly* down upon it with the tip of the finger (protected, if necessary, by a bit of cork or

gutta-percha), it must be rubbed upon the stone until the desired degree of thinness has been attained. Finally, in order to remove scratches and to polish the section, it should be rubbed upon a dry hone of very fine texture, or upon a strop charged with putty-powder. After careful washing in several waters the section must be allowed thoroughly to dry, when it may be mounted by the *dry method* in the following manner:—A ring of gold-size must, by means of the turn-table, be drawn in the centre of a slide, and the slide put away in a warm place for several days (the longer the better), in order that the ring may become perfectly dry and hard. When this has been accomplished the section is to be placed in the centre of the ring, and a covering circle of the requisite size having been cleaned, this must have a *thin* ring of gold-size applied round its margin. The cover is now to be placed in position and gently pressed down, a spring clip being employed, if necessary, to prevent it from moving. In about twenty-four hours another layer of the varnish should be applied, and the slide afterwards finished in the manner already described (§ 24). The above method is also applicable to the preparation of sections of *teeth* and also of *fruit-stones* and other hard bodies, which are incapable of being rendered soft enough for cutting.

As the process just described, however, is both troublesome and tedious, it is much better for ordinary purposes to have recourse to the *decalcifying method*, by which means sections in every way suitable for the examination of the essential structure of bone may be

obtained with ease. To carry out this plan a piece of fresh bone should be cut into small pieces and placed in a solution made by dissolving 15 grains of pure chromic acid in 7 ounces of distilled water, to which 30 minims of nitric acid s. g. 1.420 are afterwards to be added. Here they should remain for three or four weeks, or until the bone has become sufficiently soft to cut easily, the fluid being repeatedly changed during the process. From this solution they must be transferred to methylated spirit for a few days, when a piece may be selected, imbedded in paraffine, and cut in the microtome (§ 12). Some of the sections should be mounted, unstained, in spirit. For this purpose a cell of gold-size, as above described, must first be prepared and filled *full* of a mixture of spirit of wine one part, and distilled water three parts. Into this the section must be carefully placed and the cover applied, the same precautions for the exclusion of air-bubbles being taken which were recommended when speaking of mounting in glycerine (§ 16). When the cover is in position a ring of gold-size must be laid on, repeated when dry, and the slide afterwards finished in the ordinary manner. It will also be advisable to stain some of the sections with carmine (§ 14), or picro-carmine (§ 42), and mount them in glycerine. Teeth may also be treated by the decalcifying method, but in this case it must be remembered that the enamel will dissolve away.

27. *Brain*.—The best hardening fluid is that recommended by Rutherford, and is made by dissolving 15 grains of pure chromic acid and 31 grains of crys-

talized bichromate of potash in 43 ounces of distilled water. Small pieces of brain, which have previously been immersed for twenty-four hours in rectified spirit, should be placed in about a pint of this solution, where they must remain for five or six weeks, the fluid being repeatedly changed during the process. If by this time they are not sufficiently hard the induration must be completed in alcohol. Sections are easily cut in the microtome by the paraffine method (§ 12). These may advantageously be stained in a solution of aniline blue, made by dissolving 1½ grain of aniline blue in 10 ounces of distilled water, and adding 1 drachm of rectified spirit (*Frey*). As this stain acts very rapidly two or three minutes' immersion will generally be found long enough. The sections must then be mounted in balsam (§ 23).

28. *Cartilage*.—The method to be employed in the preparation of cartilage will entirely depend upon the nature of the staining agent, to the action of which the sections are to be submitted. Thus, if the elegant *gold method* is to be followed, it is necessary that the cartilage should be perfectly fresh; whilst if any of the other staining agents are to be employed the tissue may have been previously preserved in alcohol. An excellent object on which to demonstrate the gold process is to be found in the articular cartilage of bone. It is a very easy matter to obtain from the butchers the foot of a sheep which has just been killed. The joint is to be opened, and the bones dissociated, when they will be seen to have their extremities coated with a white glistening membrane—this is the *articular cartilage*.

Exceedingly thin slices must be at once cut from it, and as only small sections are required, a sharp razor may be used for the purpose, the blade being either dry or simply wetted with distilled water. The sections as cut are to be transferred to a small quantity of a half per cent. solution of chloride of gold in a watch glass. Chloride of gold may be purchased in small glass tubes hermetically sealed, each tube containing 15 grains, and costing about 2s. If, however, the student requires only a small quantity of the staining fluid he need not be even at this small expense, for as photographers for the requirements of their art always keep on hand a standard solution of chloride of gold of the strength of one per cent, a little of this may readily be obtained, and diluted to the required degree. After the sections have been exposed to the action of the staining fluid for about ten minutes they may be transferred to a small beaker of distilled water, and exposed to diffused light for about twenty-four hours, when they must be mounted in glycerine (§ 16).

Sections of cartilage may also be examined, without being stained, in which case the field of the microscope should be only very feebly illuminated. Or carmine staining (§ 14) may be resorted to—these sections show well in glycerine, or if the staining be made very deep, even Canada balsam may be employed, and with fair results.

Microscopists are indebted to Dr. Frances Elizabeth Hoggan for the description of a new method of staining, which we have found especially suited to the treatment of cartilage. The agent employed is *iron*,

and the process, which is very simple, is as follows. Two fluids are necessary—(1) tincture of steel ; (2) a two per cent solution of pyrogallic acid in alcohol. A little of the former is to be poured into a watch glass, and into this the sections, after having been previously steeped in alcohol for a few minutes, are to be placed. In about two minutes the iron solution is to be poured away and replaced by solution No. 2. In the course of a minute or two the desired depth of colour will have been produced, when the sections are to be removed, washed in distilled water, and mounted in glycerine. The results obtained by this process are very beautiful, the colour produced being a very fine neutral tint, of delightful softness. The process also answers admirably in the case of morbid tissues, and we have now in our possession some sections of ulcerated cartilage tinged by the iron method, in which the minute changes resulting from the ulcerative disintegration are brought out with wonderful distinctness.

As the structure of cartilage differs according to its purpose and situation, the student will find his time profitably employed in a careful examination of the following forms (α) *hyaline*—articular and costal ; (β) yellow fibro-cartilage—epiglottis, or external ear ; (γ) *cellular*—ear of mouse. Sections of the *intervertebral ligaments* should also be made, in which the different kinds of cartilage may be examined side by side with each other.

29. *Coffee Berry* affords sections of great beauty. The *unroasted* berry should be soaked for hours or days in cold water until sufficiently soft ; then imbedded in

paraffine, and cut in the microtome (§ 12), the section being made in the direction of the long axis of the berry. Put up in glycerine, or stain rather strongly with carmine, and mount in balsam. The same method of treatment may also be applied to other hard berries or *seeds*.

30. *Fat*.—Adipose tissue may be hardened in alcohol, cut in paraffine, and mounted in glycerine. If the tissue has been injected the sections may be mounted in balsam, and are then very beautiful objects, showing the capillary network encircling the fat cells.

31. *Hair*.—Longitudinal sections are readily made by splitting the hair with a sharp razor. It is more difficult to cut the hair transversely. This, however, may easily be done in the following manner. The hairs having previously been well soaked in æther to remove all fatty matters, a sufficient number of them must be selected to form a bundle about the thickness of a crow quill. This bundle, after being tied at each extremity with a bit of thread, is to be immersed for several hours in strong gum (§ 18,) to which a few drops of glycerine have been added. On removal, the bundle must be suspended by means of a thread attached to one end of it, in a warm place until sufficiently hard, when it is to be imbedded and cut in paraffine (§ 12). Each section, as cut, is to be floated off the knife into methylated spirit. From this it is with the aid of the spoon (§ 14) to be transferred to a slide, the spirit tilted off, a drop of absolute alcohol added, when, after a minute or two, this also is to be drained off, the

section treated with clove oil, and the mounting completed as described in § 23.

32. *Horn* varies very much in consistence, in some instances having a cartilaginous character, whilst in others it is almost bony. In the latter case, sections will have to be ground down in the manner explained when speaking of bone (§ 26). Where the texture is less dense, recourse may be had to prolonged steeping in hot or boiling water; in some cases it will be necessary to continue the immersion for several hours. When sufficiently soft the piece of horn may, by means of bits of soft wood, be firmly wedged into the tube of the microtome, and sections cut with a razor, or what is better, with a broad and very sharp chisel. The sections are to be put between glass slips, held together by American clips (or pegs), and put away for two or three days in order to become thoroughly dry. After well soaking in good turpentine or benzole, they must be transferred to slides, the superfluous turpentine drained off, and chloroform-balsam added, etc. (§ 23). Sections of horn should, of course, be cut in different directions, but for examination with the polariscope those cut transversely yield by far the most magnificent results. *Hoofs, whalebone*, and allied structures should also be treated by the above method.

33. *Intestine*.—The method to be pursued with *sections* has already been described (§ 18). The ileum, however, is a very pretty object when a portion of it is so mounted as to show the *villi erect*. To do this it is necessary to cement to the slide, by marine glue, a glass cell of sufficient depth. This should have been pre-

pared some time beforehand, so that the cement may be perfectly dry and hard. The cell is now to be filled with turpentine, and the piece of ileum (having been previously passed through methylated spirit and absolute alcohol into turpentine) is gently placed into it, having the villi uppermost; pour some pure and rather fluid balsam on the object at one end, and gradually incline the slide, so as to allow the turpentine to flow out at the opposite side of the cell, till it is full of balsam. Then take a clean cover, and having placed upon it a small streak of balsam from one end to the other, allow it gradually to fall upon the cell, so as to avoid the formation of air-bubbles (§ 17), and finish the slide in the usual manner.* Or, the intestine may be dried, and mounted *dry*, in a cell with a blackened bottom, for examination as an opaque object.

34. *Liver*.—Small pieces of liver may be very successfully hardened by immersion in alcohol, beginning with weak spirit and ending with absolute alcohol. Cut and mount as usual.

35. *Lung* must be prepared in chromic acid (§ 5). For the cutting of sections the freezing microtome (§ 18) is of especial value, and should, therefore, be used. If, however, the student be not provided with this instrument, he must proceed as follows. A small piece of lung, previously deprived of all spirit, is to be immersed until thoroughly saturated in solution of gum (§ 18). A small mould of bibulous paper (§ 2), only just large enough to receive the piece of tissue, having been prepared and filled with the mucilage, the speci-

* Ralf.

men is to be transferred to it. The mould, with its contents, is now to be placed in a saucer, into which a mixture of about 6 parts of methylated spirit and 1 part of water (*Schafer*) is to be poured until the fluid reaches to within about a third of the top of the paper mould. In the course of several hours the surface of the mucilage will begin to whiten and solidify. As soon as this occurs more dilute spirit must be poured into the saucer, until the mould is completely submerged. In a day or two the gum will be found to have acquired a suitable consistence for cutting, when it must be removed from the spirit, the paper mould peeled off, and the mass imbedded and cut in paraffine, the sections being afterwards treated as if they had been obtained by the freezing method (§ 18). If the solidification of the gum should proceed too slowly, a few drops of pure spirit may be added to the contents of the saucer. If, on the other hand, the gum should become overhard, it will be necessary to put into the saucer a few drops of water, and repeat this until the required consistence be obtained.

36. *Muscle*.—Harden in chromic acid, and cut in paraffine. Transverse sections may be made to show the shape of the fibrils. Longitudinal sections will only be required in the case of injected tissues, when such sections will be found very elegant, showing, as they do, the elongated meshes of capillaries running between and around the muscular fasciculi. Mount in glycerine or balsam. To see the transverse striæ characteristic of voluntary muscle, a very good plan is to take a bit of pork (cooked or fresh), and by means of needles to

teaze it out into the finest possible shreds. If these be examined in water or glycerine, the markings will be shown very perfectly.

37. *Orange-peel*, common object though it be, is not to be despised by the microscopist. Transverse sections must be prepared by the gum method (§ 35). These sections are not to be subjected to the action of alcohol (as this would destroy the colour), but after drying between glass slides they must be soaked in turpentine and mounted in balsam. We shall then have a good view of the large globular glands whose office it is to secrete that essential oil upon which the odor of the orange depends.

38. *Ovary* may be prepared in the same manner as liver (§ 34). Sections, which are to be cut in paraffine, may be stained with carmine, and mounted in glycerine or balsam. Apart from all scientific value, we know of no slide for the microscope which, even as a mere object of show, surpasses in beauty a well-prepared section of *injected ovary*, showing the wondrous Graafian vesicles, surrounded by their meandering capillaries.

39. *Porcupine Quill*.—Soften in hot water, cut in paraffine, and mount in balsam. Much (in our opinion *too much*) lauded as an object for the polariscope.

40. *Potato*.—From the large amount of water which it contains thin sections cannot be cut from the potato in its natural state. It must, therefore, be partially desiccated, either by immersion in methylated spirit for a few days or by exposure to the air. Sections may then readily be obtained by imbedding and cutting in paraffine. Such sections mounted in balsam are very

beautiful, the starch being seen *in situ*, whilst if polarized light be employed each granule gives out its characteristic black cross.

41. *Rush* is to be prepared and cut as orange-peel (§ 37). Transverse sections of this "weed" furnish slides of the most exquisite beauty.

42. *Skin*.—To prepare skin for section a piece is to be selected which, after having been boiled for a few seconds in vinegar, must be stretched out on a bit of flat wood, and being maintained in position by pins be allowed to remain until thoroughly dry. Then imbed in paraffine, and cut *exceedingly* thin transverse sections. These may be stained in carmine, but more beautiful results are obtained if picro-carmine be employed. Sections of skin, when stained by this agent are much increased both in beauty and instructiveness; for the several constituents of the tissue becoming tinged with different colours are readily distinguishable from each other, whilst the contrast of colouring forms a pleasing picture to the eye. The method of preparing picro-carmine is very simple, though it sometimes yields a solution not altogether satisfactory. The best formula with which we are acquainted is that given by Rutherford,* and if due care be taken in following it out failure will generally be avoided. "Take 100 c.c. of a saturated solution of picric acid. Prepare an ammoniacal solution of carmine, by dissolving 1 gramme in a few c.c. water, with the aid of excess of ammonia and heat. Boil the picric acid solution on a sand bath, and when boiling add the carmine solution. Evaporate

* "Practical Histology," 2d edit. p. 173.

the mixture to dryness. Dissolve the residue in 100 c.c. water, and filter. A clear solution ought to be obtained; if not, add some more ammonia, evaporate, and dissolve as before." Sections may be exposed to the action of this fluid for a period varying from fifteen to thirty minutes, then rapidly washed in water, and mounted in glycerine. They may also be mounted in balsam, care being taken in that case to shorten as much as possible the period of their immersion in alcohol, so that no risk may be run of the picric acid stain being dissolved out.

If it is intended to study the structure of the skin with anything like thoroughness, portions must of course be examined from different localities, in order that its several varieties and peculiarities may be observed. Thus the *sudoriferous*, or sweat glands, may be found in the sole of the foot, whilst the *sebaceous* glands are to be sought in the skin of the nose. The *papillæ* are well represented at the tips of the fingers,* whilst the structure of the shaft of the *hair*, together with that of the follicle within which its root is enclosed, as also the muscles by which it is moved, are to be studied in sections of skin from the scalp or other suitable locality.

43. *Spinal Cord.*—The spinal cord, say of a cat or

* It is well, in connection with these papillæ, to bear in mind a fact pointed out by *Frey*, namely, that the tips of the fingers frequently become, *post-mortem*, the seat of extensive natural injections; hence, in sections from this region, we frequently obtain good views of distended capillaries without having been at the trouble of previously injecting them.—*Frey*, "Microscopical Technology."

a dog (or if procurable, of man), after being cut into pieces about half an inch in length, may be hardened in the usual chromic acid fluid (§ 5). As it is peculiarly liable to overharden and become uselessly brittle, the process must be carefully watched. Its further treatment is the same as that of brain. These sections may be stained very satisfactorily by the *ink process*, for communicating details of which we are indebted to the kindness of Dr. Paul, of Liverpool. The agent usually employed is Stephenson's blue-black ink, which, for this purpose, must be quite fresh. As in the case of carmine, two methods of staining may be adopted—either rapid, by using concentrated solutions, or more prolonged, according to degree of dilution. For the reasons previously given (§ 14), slow methods of staining are always to be preferred, as yielding the most beautiful results, yet, for the purposes of preliminary investigation, it is often convenient to have recourse to the quick process. To carry out the latter plan, an ink solution of the strength 1 in 5—10 parts of water is to be freshly prepared, and the sections exposed to its action for a few minutes. For gradual staining the dilution must be carried to 1 in 30—50, and the time of immersion prolonged to several hours, the sections being occasionally examined during the staining, so that they may be removed just as they have acquired the desired tint. When a satisfactory coloration has been obtained, the preparations should be mounted in dammar or balsam (§ 23). One advantage of this method of staining is, that definition is almost as good by artificial light as by day.

44. *Sponge* may readily be cut after being tightly compressed between two bits of cork; or its interstices may be filled up by immersion either in melted paraffine (§ 11) or in strong gum (§ 18), and then cut as usual.

45. *Stomach* requires no special method of hardening (chromic acid). Sections should always, when practicable, be cut in the freezing microtome. In default of this, proceed in the manner as directed for lung (§ 35). Both vertical and horizontal sections will, of course, be required. If the preparation has been injected, the latter are particularly beautiful. Stain with carmine or aniline blue (§ 27), and mount—if for very close study, in glycerine—if injected and for a “show” slide, use balsam.

46. *Tongue*.—Harden in chromic acid, imbed and cut transverse sections in paraffine. As, however, the paraffine is apt to get entangled amongst the *papillæ*, whence it is afterwards with difficulty dislodged, it will be as well before imbedding to soak the tongue in strong gum for a few minutes, and afterwards immerse in alcohol till the gum becomes hardened, so that the delicate *papillæ* may thus be protected from the paraffine by a surface-coating of gum. The best staining agent is picro-carmine (§ 42). Sections of *cat's* tongue near the root, when thus stained, furnish splendid objects. Sections should also be made of the *taste-bulbs*, found on the tongues of rabbits. These are small oval prominences, situated one on each side of the upper surface of the tongue near its root. They should be snipped off with scissors, and vertical sections made in

the direction of their long axis. Stain with carmine or picro-carmine, and mount in glycerine or balsam.

47. *Vegetable Ivory*—After prolonged soaking in cold water may readily be cut in the microtome. The sections should be mounted in balsam, and though not usually regarded as polariscope objects, nevertheless, when examined with the *selenite*, yield very good colours.

48. *Wood*.—Shavings of extreme thinness may be cut from large pieces or blocks of timber, by means of a very sharp plane. In this way very good sections may be procured of most of the common woods, as oak, mahogany, “glandular wood” of pine, etc. Where however, the material to be operated upon takes the form of stems, roots, etc., of no great thickness, they should, after having been reduced to a suitable consistency (§ 4), be imbedded in paraffine, and cut in the microtome. Before imbedding it must not be forgotten to immerse the wood to be cut in weak gum-water (§ 11), this precaution being of great importance, especially in the case of stems, etc., the bark of which is at all rough and sinuous. If the sections are to be mounted *unstained*, they are usually put up in weak spirit (§ 26). A very general method also of dealing with this class of objects is to mount them *dry* (§ 26). This plan, however, cannot be recommended, for however thin the sections may be, the outlines, when this process is adopted, always present a disagreeable black or blurred appearance. To avoid this we may have recourse to Canada balsam, but the ordinary method of employing it must be slightly modified, a drop of chloro-

form being substituted for the clove oil (§ 23), otherwise this latter agent will cause the section to become so transparent as to render minute details of structure difficult to recognize. A better plan, perhaps, is to stain the section with carmine or logwood, and mount in balsam by the ordinary process. The best course to follow, however, especially in the case of transverse sections, is the *double staining* method.* For this purpose the sections in the first place must be subjected to the action of a solution of chloride of lime ($\frac{1}{2}$ oz. to a pint of water) until they become thoroughly bleached. They must then be soaked in a solution of hyposulphite of soda (one drachm to four ounces of water) for an hour, and after being washed for some hours, in several changes of water, are to be transferred for a short time to methylated spirit. Some red staining-fluid is now to be prepared by dissolving half a grain of Magenta crystals in one ounce of methylated spirit. A little of this solution being poured into a small vessel of white porcelain (§ 14); the sections are to be immersed in the dye for about thirty minutes. They are now to be removed, and after *rapid* rinsing in methylated spirit to remove all superfluous colour, they must be placed in a blue staining fluid made by dissolving half a grain of aniline blue in one drachm of distilled water, adding ten

* See a paper by Mr. Stiles in the "Pharmaceutical Journal;" also "Monthly Microscopical Journal" for August, 1875. [For a very exhaustive paper on this subject by the late Dr. Beatty, of Baltimore, Md., see "American Journal of Microscopy" for June, 1876.

minims of dilute nitric acid and afterwards sufficient methylated spirit to make two fluid ounces. The sections must be permitted to remain in this solution for a very short time only, one to three minutes being generally sufficient, for as the action of the dye is very energetic, it will, if too long exposure be allowed, completely obliterate the previous coloration by the magenta. After being again *rapidly* rinsed in methylated spirit, as much of this as possible must be drained off, and the sections put into oil of *cajeput*, whence, in an hour, they may be transferred to spirits of turpentine, and after a short soaking, mounted in balsam.

If the student will carefully carry out the above process, his trouble will be amply repaid by the beautiful results obtained, for by its means he may, with ease, prepare for himself a series of slides of such value as to constitute a worthy addition to his cabinet.

The preceding list by no means represents all the objects, sections of which will be found interesting to the microscopic student. Such was not its purpose--had it been so, the enumeration might have been prolonged almost indefinitely. The end in view was to bring under the notice of the reader only those substances the cutting of which is accompanied by difficulty; and even of this class the space at our disposal has been so limited that we have been unwillingly compelled to pass over many, and dwell only on such as possess a typical character.

NOTES.

NOTE A., Page 14.

RANGOON oil is not found in this market. It is a variety of naphtha or petroleum found at Rangoon, in Asia, and contains a variable percentage of solid paraffin. Kerosene with a very little paraffin dissolved in it, would undoubtedly answer the same purpose.

Good sperm oil or tallow (free from salt) will serve quite as well. It is scarcely necessary to caution the reader that all oily matter should be removed from the knife before it is used for cutting sections. This is best effected by dipping it in hot water and carefully wiping it.

NOTE B., Page 15.

METHYLATED spirit is alcohol which contains a small percentage of methyl. This renders it unfit for use in the manufacture of liquors or even chloroform, but does not injure it as a preservative fluid. The scientific men of Great Britain have the privilege of obtaining this fluid free from duty, and consequently they make very general use of it. In this country the student of science has no such privilege, and must use the more expensive alcohol. This however is but of a piece with other restrictions placed upon scientific pursuits in this country, where men like Marshall Jewell and Hannibal Hamlin have influence enough to pass laws which prohibit the exchange by mail of microscopic specimens, which pass freely in every other civilized country.

True methyl alcohol, or methylated spirits, is not to be had in this country, and therefore whenever this liquid is directed to be used in English books the American microscopist must substitute good alcohol. What is sold for "methyl alcohol" is wood spirit or wood naphtha, and will not serve the purposes of the microscopist, though it answers well enough for spirit lamps, making varnishes, etc.

NOTE C., Page 20.

THE method described in the text is generally known as the "free-hand" method, and although it is not generally employed for the production of cabinet specimens or slides that are intended for sale, yet it possesses so many advantages that every student should endeavor to acquire the art of cutting sections without the use of the microtome. For purposes of study such sections are quite equal to the best of those usually found on sale, and for the encouragement of beginners we may add that we have seen sections cut by the free-hand method which for size, thinness and accuracy, excelled any that we have ever seen produced with the microtome. Indeed Schaeffer distinctly affirms that no microtome can equal the skilled hand in the production of thin sections.

NOTE D., Page 22.

THE mere existence of a binding screw should be no objection, and where bushings are employed it is sometimes necessary. But as the author well observes, a binding screw cannot be used to secure histological preparations.

NOTE E., Page 22.

A STILL better system is where the section knife is held in a frame which slides on the plate so that the edge is kept just above the surface. In this way the edge of the knife is never dulled by grinding on glass or metal. A common mistake in this form, however, is to raise the cutting edge too far above the surface of the plate. This gives rise to great irregularities.

NOTE F., Page 22.

As the size of the sections which it may be desired to cut, varies within wide limits, the best microtomes made in this country are furnished with as many as three tubes or "bushings," fitting one within the other, and in this way the hole may be partially filled up and thus reduced in size. This enables us to obtain a range of from one inch and a quarter, suitable for large histological preparations, to one-eighth of an inch for such objects as hair sections.

NOTE G., Page 37.

KNIVES FOR CUTTING SECTIONS.

SUCCESS in cutting sections depends more upon the excellence of the knife that is used than upon any other one point, and therefore the art of keeping the knife in good order is one which should be acquired by every microscopist, even at the cost of considerable time, study and labor. As a stepping stone to the acquisition of this art, there are certain general principles which must be thoroughly understood before we can hope for success in practice. These principles are simple, and when once clearly stated, quite obvious, but they are frequently overlooked.

Cutting instruments act in two ways — either as simple wedges or as a series of wedges, the latter being generally known as the saw-like action. It is frequently stated in school philosophies that the edges of *all* cutting instruments are toothed like a saw, and that they act like a saw; this is evidently not the case with many, such as the axe, the chisel, the plane, etc., and it is not even true of several to which it is frequently applied, such as the razor, the penknife, etc. The action can be said to be "saw-like" only when the teeth act as independent wedges and remove a small portion of the substance acted upon, the portion being known as *saw-dust*. Now in cutting sections of wood with a sharp razor, where but a single stroke is used to carry the cutting edge across a section, say half an inch in

diameter, the edge of the razor does not act like a saw; none of the material is removed except the section itself; in other words, there is no saw-dust. That the edge of the knife or razor is not even, is very easily proved by means of the microscope, but it will be found that the notches and projections form a series of lancet-shaped knifelets, each one of which acts just as does the blade of a penknife when drawn over a piece of wood or a quire of paper. Now if we lay a quire of paper on a board and draw the edge of a penknife over it, we will cut down through the paper, not by sawing but by a moving wedge. It is precisely in this way that the little lancet-shaped teeth on the edge of a razor act when the latter is used to cut a section.

If we further examine the edge of the razor, we will find that the bottoms of the notches are comparatively blunt; if therefore we press the razor directly forward we soon press these blunt portions of the general edge against the material to be cut, and the resistance becomes so great that the material is actually crushed or torn. This would not happen if we had an absolutely perfect edge—one without any notches. This being practically unattainable, however, we must endeavor to make up for it by a sawing motion, (though not a sawing action) at the same time seeking to avoid as much as possible those saw-like features which render this motion necessary. We have dwelt at length upon this point because so much has been written comparing the edge of a razor to that of a saw, that some persons actually advise us to give the edges of our knives and razors a saw-like character, under the impression that by so doing they will work better. This is a fallacy; the best work will be obtained from the smoothest and most perfect edge, provided the latter is thin enough.

If the material of which our cutting instruments are made would allow it, that is to say if it was perfectly hard, perfectly rigid and incapable of being crushed or torn apart, the proper shape of a cutting instrument would be a wedge of the most acute form possible. But since the steel which we use is limited in its hardness and its power of resisting crushing and

bending influences, the blades of our knives must have a certain strength or thickness, depending upon the character of the material to be cut. For very hard substances the knife must be stout and the edge ground to a comparatively obtuse angle; for moderately soft substances, such as wood, the angle may be more acute, while for very soft tissues the thinnest blade and the sharpest edge are most suitable.

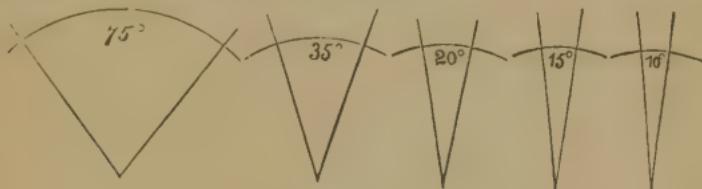


Fig. 1.

The various angles to which cutting tools of different kinds are ground are shown in the accompanying illustrations, which explain themselves.

In the diagrams just given, the sides of the wedge are carried in straight lines to the very edge. In practice, however, this is never done, there being two strong objections to such a form. In the first place if the sides were as shown in the figures, every time the tool was ground or set, the entire side would have to be ground off, and this would involve great

labor. But another difficulty, quite as great, would occur in the use of the instrument, for since the wedge keeps growing constantly and proportionately thicker, the friction between the sides of the wedge and the material would rapidly become very great. Therefore most cutting edges are ground to a second angle, much more obtuse than the first, and it is this second angle which forms the true cutting edge. And it

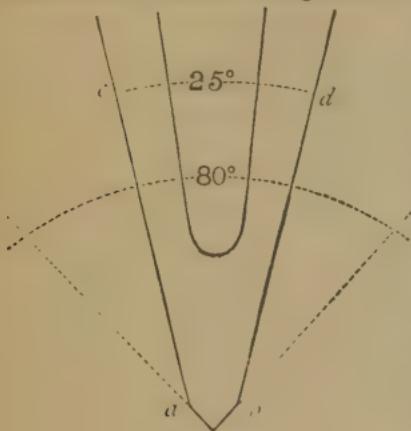


Fig. 2.

is obvious that the mass of material required to carry out the ultimate angle in any case is wholly unnecessary, for if the tool be strong enough at *a b* (Fig. 2) it will certainly be strong enough at *c d*. An extreme illustration of this principle is shown in the cold chisel used for cutting iron, the edge of which is shown in Fig. 2, and the angle of 80° to which it is ground is carried out by dotted lines. The angle which the sides of the chisel (not the sides of the extreme cutting edge however) make with each other is 25° .

There are two methods by which this change of angle may be made, one of which is shown in the cold chisel just figured, and the other in the razor of which a section is shown in figures 3 and 4. In the case of the cold chisel it will be seen that the tool is first formed to a thin straight wedge which is afterwards changed to one that is much more blunt. This answers very well where the ultimate angle is comparatively large or blunt, as is the case with penknives, table-knives, carving-knives, etc. But where the ultimate angle is very small this plan does not answer well, and the method shown in Fig.

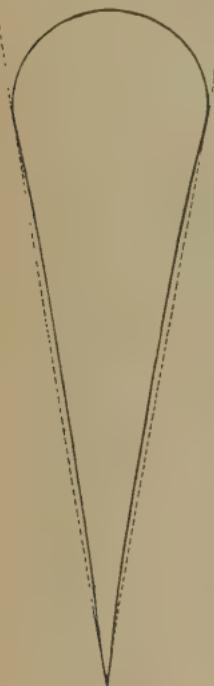


Fig. 3.

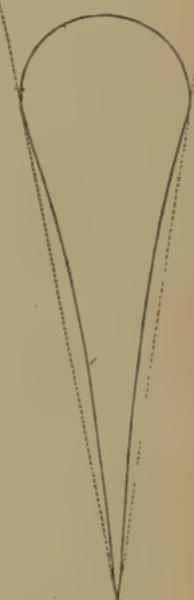


Fig. 4.

3 is generally adopted. Here the ultimate angle is such that lines touching the extreme edge and the back of the blade are perfectly straight and form the actual cutting angle. In this case, therefore, the relief is obtained by hollowing out the sides of the blade, and this is done to various extents, the extremes being shown in figures 3 and 4. Fig. 3 shows a section of a razor

ground on a stone 12 inches in diameter, which is as large as is generally used for this purpose. Fig. 4 is a section of a razor ground on a four-inch stone—the smallest in general use. This method of changing the angle is of course substantially the same as the first, merely differing in the mechanical device used, but it affords this important advantage that in the subsequent honing and stropping processes the back of the razor forms a perfect guide by which the ultimate angle may be determined. This is not the case with the cold chisel or the carving-knife, in both of which cases the eye and hand alone determine the cutting angle, which is therefore apt to become irregular or even rounded—the worst form of all. This will be more easily understood from the following engravings where A, Fig. 5, shows a pen-knife blade, as applied to an oil-stone for the purpose of giving it the final edge. The angle here shown is considerably

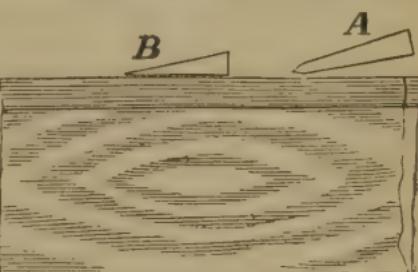


Fig. 5.

greater than that generally used, but the principle is the same. It is evident that if, in moving the blade back and forth on the stone we allow it to rock or change the angle which the blade makes with the stone's surface, the edge will become rounded as shown in Fig. 6—a form which for delicate work is useless.



If the blade be laid down flat as at B, Fig. 5, the labor of wearing away the superfluous metal will be enormous. But if the blade be hollow as Fig. 7, (where the hollow is, for clearness of illustration, slightly exaggerated) then the ultimate

Fig. 6. angle may be formed quickly and accurately, there being no danger of the angles being changed on account of want of skill on the part of the operator.

In some cases one side is left quite flat and the other side is ground to two or sometimes three angles, as in the carpenter's chisel, a section of which is shown in Fig. 8, where $\alpha\alpha$ shows the

angle made by the body of the blade; *b b* the angle of the bevel formed by the grindstone, and *c c* the angle of the cutting edge which is formed by the oilstone. In this case the flat side

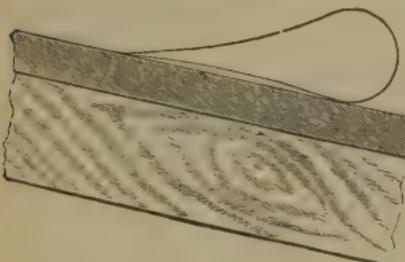
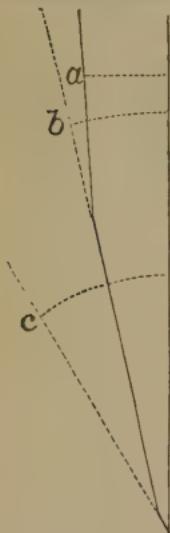


Fig. 7.

A B forms a straight guide and enables the workman, in cutting, to make straight, clean work. In sharpening such a tool on the oilstone the flat side is always laid flat on the oil-stone and merely smoothed off,* and the skill of the workman is chiefly shown in the

accuracy with which he forms the angle *c B c*. The dexterity with which skilled workmen can effect this by the hand alone



is astonishing, but in the hands of those who have had but little practice the edge infallibly *a* becomes rounded as shown in Fig. 6. Therefore *b* wherever a knife with a flat side is used, no attempt should ever be made to grind this flat side *A* on the hone. Such an attempt will almost always result in an edge so irregular that it will be *c* almost impossible to cut a good section with it. But if the under side of the knife be left truly flat, we will have the very best tool that can be had for use with the microtome. Unfortunately, however, it is very difficult to get a knife with a truly flat surface, that is, one that is perfectly "out of wind" as mechanics say. As a general rule the flat sides of knives are not true planes but

Fig. 8. irregularly curved surfaces; consequently when laid on a plane surface, such as the table of a microtome, they rock, and the edge is in contact with the table at some points and separated from it at others, and the points which

* Holtzapffel tell us that chisels that are required for paring across the end grain of moderately soft wood are considered to hang better to the work when they have a very slight keen burr or wire edge thrown up on the face or flat side of the tool. But this does not apply to section knives.

are in contact change as the knife is moved diagonally across the table. This makes the section irregular and worthless. But a really good knife with a perfectly true flat side is such a valuable tool that a good deal of effort may be profitably expended in getting it, and when once obtained never let the flat side be touched by a grindstone, or more than touched by a hone. It is not absolutely necessary, however, that the sides of a delicate knife should be curved, provided the knife is used for cutting very soft materials. An angle of 10° or even 5° gives a pretty stout blade when carried back three-quarters of an inch, as may be seen by examining Fig. 1, and the guiding action of the very thick back may be obtained by means of an artificial guide, applied like the brass backs of tenon saws. Such an arrangement is shown in Fig. 9, and has been found very effectual.

When the knife

is to be honed, the back is applied and fastened by means of two or three set screws. When laid on the hone, the edge of the knife

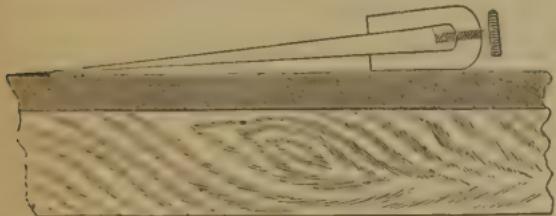


Fig. 9.

and the lower surface of the back form the guide and regulate the cutting angle. In this way we can use a broad, thin blade and yet secure great accuracy in honing it. Such a blade has this advantage also, that it is not so liable to be rounded and thus injured by stropping as one in which the relief is obtained by hollowing out the sides.

Such, then, are the general principles which govern the construction of cutting tools, including the section-knives used by microscopists. We will now give a few practical directions for selecting a knife and putting and keeping it in order.

In selecting a knife for cutting sections, regard must be had to the texture of the material that is to be cut. To attempt to cut delicate sections of soft tissues with the stout knives which are suitable for cutting sections of woody fibre would be to de-

stroy the sections, while to reverse the operation and cut sections of wood with thin, delicate knives would result in the ruin of the knife. We have seen a most excellent knife seriously injured by an attempt to cut material that was too hard for it. The knife was very thin, and had proved most excellent in cutting sections of such material as kidney, liver, etc. An attempt was made, against our protest, to cut a section of an apple-shoot, the wood of which was mature. Before the knife had gone half way across, it bent, dug into the wood and broke, leaving an ugly gap in the middle. Experienced section cutters know this very well, but young microscopists are not so familiar with the facts just stated, and the point is too important to be overlooked. Those, therefore, who devote themselves to microscopical studies, or who expect to make sections of materials of several kinds, differing in hardness, etc., must provide themselves with knives of different degrees of strength.

For common work, good razors are as good as anything, provided they can be obtained with straight edges. Where razors are not suitable, recourse must be had to the surgical instrument maker, though we are sorry to say that there are but few in this country that know how to forge, temper and grind a decent knife. Most of our dealers in instruments do not make the instruments they sell; they import the goods they sell with their names stamped on, and thus get a reputation as manufacturers; a special order they are unable to fill respectably. There are some exceptions, but of the majority of dealers what we have written above is true.

The points which specially demand attention in a knife for cutting sections are these: 1. Quality of the steel used; 2. Temper; 3. Form of the blade.

Of the quality of the material of which a knife is made, nothing can be determined except by actual trial. The old tests of staining with acids, examining with the microscope, etc., are worthless, or at least too crude and uncertain to be of any practical value. Color changes with the degree of polish

that is produced, and, in short, there is no reliable guide. The purchaser must depend entirely upon the reputation of the manufacturer. There is plenty of good steel to be found; the trouble lies with the cutlers. They are careless and in haste, and as a consequence they burn the steel or fail to work it sufficiently, and the result is a useless tool.

The steel may be of the very best quality, however, and well forged, and yet the knife may fail from being badly tempered—too soft or too hard. If too soft, the edge is soon dulled; the knife requires to be frequently honed, and the time wasted in keeping it in order is a serious drawback. If too hard, it is impossible to give it a keen edge, for the metal crumbles away as soon as it is honed or stropped very thin, and the edge becomes ragged and dull. Good steel, well forged, may be so tempered that it will neither crumble nor become rapidly dull.

Much may be learned on this point from careful inspection of the edge, and trial on a piece of horn, such as an old razor handle. When drawn over a clean piece of horn once or twice the edge of a soft knife is completely dulled; if well-tempered it should scarcely lose its keenness. Again, when laid flat on the thumb nail and pressed, the edge ought to bend up without breaking or crumbling, and at once, when the pressure is removed, resume its original shape. The extent to which extreme hardness and durability, or toughness, may be combined, is well shown in the famous Toledo sword-blades. One of these blades will shear through an iron nail without having its edge perceptibly dulled, and yet so tough and springy is it that it may be coiled up into a hoop of several folds without breaking or receiving any permanent set. If we could only get section knives of stuff like this, it would be a pleasure to work with them.

After all, however, the only test of these two points, material and temper, is a fair trial in actual practice. Of the form of the blade, however, it is easy to judge, and there are a few points which are frequently overlooked and which give rise to errors that are attributed to other causes.

In the text we are told that "It is essentially necessary that the back and edge of the blade be strictly parallel to each other." The author undoubtedly knew what the correct form should be, but the definition he has given is not a correct mathematical statement of the conditions involved. These are as follows:

The edge must form a straight line, and both the edge and the under side of the back must lie in the same plane.

Let us consider these conditions somewhat carefully.

Since the hole in which the material to be cut is imbedded, is always circular, and since the blade is generally pressed forcibly against the table of the microtome, it is obvious that if the edge of the knife be curved it will sink into the hole to a greater or less depth according as it is further from the centre of the

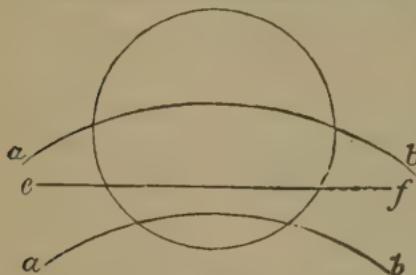


Fig. 10.

hole or nearer to it. To explain this let us refer to the greatly exaggerated drawing given in figure 10 where the edge of the knife, *a*, *b*, is shown in two different positions covering the hole of the microtome. The least degree of reflection will show that as the

knife passes towards the centre from *a b* to *a' b'*, it will have a tendency to turn on the back as on a hinge, and the middle of the edge will sink deeper into the hole as it approaches the centre of the latter. The section will therefore vary in thickness and prove useless. This difficulty can be obviated only by the use of a straight edge like *c f*, which will always lie in the same plane.

If the knife were perfectly rigid, and the curved edge and back lay entirely in the same plane, this difficulty would not occur to any great extent. But since a curved edge rarely lies wholly in the plane of the back of the knife, and since the blades of most knives, especially those thin ones used for cutting sections of soft tissues, yield a little to pressure, and since

this pressure is never exactly the same, it is impossible to prevent irregularities from occurring.

While it is not necessary that the back of the knife and the cutting edge should be parallel to each other, it is absolutely necessary that they be in the same plane, or, as mechanics say, —“out of wind.” If this condition be not observed, the blade will rock on the microtome table as it passes over it, and irregularity in thickness of section will result.

A slight knowledge of geometry will enable any one to see that these statements are strictly accurate.

As regards other points in the form of the knife, we refer to what has been previously said when discussing the general principles which should govern the construction of cutting tools.

We now proceed to give a few practical directions for sharpening knives and razors and keeping them in order.

Section knives, during the process of sharpening, are subjected to three distinct processes: 1, Grinding; 2, Honing; 3, Stropping.

Grinding is one of the most important, though it is probable that it will rarely be undertaken by the microscopist himself. More knives and razors are spoiled in grinding than in any other way. We have now in our possession an excellent knife, which in an evil hour we entrusted to a New York cutler, and received it back utterly ruined, the temper being entirely taken out of the blades! Some of our readers may wonder at this, but unfortunately it is too true. The cutler, to save time and trouble, too frequently holds the blade against the stone with such force that it becomes over-heated, especially when he is a little careless and allows the supply of water to fall short.* The owner does not find this out until he discovers that the knife is ruined, because nothing is more easy than to cool the blade and grind off the tell-tale blue spots. When charged with his rascality, the cutler always denies it and lays the blame

* Nothing is more easy than to heat a thin rod or stout wire red hot by holding it against a dry grindstone rapidly revolving. We have often kindled fire in this way.

on a "soft spot in the steel"—a miserable subterfuge—too transparent to deceive the least experienced.

The only protection against this is either to entrust the knife to a man of known carefulness and integrity, or to stand over him while he does it. If the knife be ground under the owner's eye, no fear need be entertained, because it is easy to insist upon the use of plenty of water.

The grindstone used for section knives should be of fine grain, and it should run true and be very straight across the face, otherwise it will be impossible to grind the knife true, and this, as we have seen, is a necessity.

Every microscopist that expects to do much at cutting sections must learn to hone his own knife or razor. Of the various hones in use the famous Turkey oilstone is said to be altogether the best. It is, however, very difficult to get it genuine and in sufficiently large pieces. We therefore use the German hone-slate, a softer, yellow stone of wonderful fineness and cutting power. The ordinary whetstones are altogether too coarse, and as for artificial stoues and strops, they may do for coarse work and common razors for *scraping*, but they will not do for knives used for section cutting. If any of our readers should be so fortunate as to possess a good, old-fashioned Turkey oil-stone with a fair sized surface, let them think much of it. It is invaluable. Years ago, when working on some investigations that required the use of very sharp knives, we were offered, by an old English carpenter, the use of a real Turkey stone. We have never since found anything that did its work so quickly and so well.

To merely mention the substitutes for Turkey oilstone would fill some pages. We therefore confine ourselves to the German hone. It is obtained from the slate mountains in the neighborhood of Ratisbon, where it occurs in the form of a yellow vein running vertically into the blue slate, sometimes not more than an inch in thickness, and varying to twelve to eighteen inches. After being quarried it is sawed into thin slabs, which are usually cemented to a similar slab of the blue

slate as a support, and the combined stones are then set in a wooden frame and fitted with a cover, also of wood. Unlike the Turkey oilstone, the Arkansas stone, and some others, the German hone is soft and easily scratched and worn. It must therefore be carefully preserved, and as the *flatness* of the hone is an important point, it should never be used for anything but section knives.

Having purchased a hone, the first thing to do is to see that it is perfectly true; that is, that its surface is a perfect plane. If hollow or twisted, it is useless until made straight. It may be tested by means of two steel or wood straight-edges, and the method of doing this, which is very simple but difficult to describe, the microscopist can learn from any cabinet maker or carpenter. If the hone be not true it must be made so, and this is best done by grinding it with emery on a slab of marble or, better still, a plate of cast iron which has been planed true. (Waste castings with planed surfaces may be found in most machine shops, and may be either borrowed or purchased for a small sum.) If the hone is very uneven, coarse emery may be used at first; then finer must be taken, and so on until the surface of the hone is not only true but very smooth. This involves considerable labor, but it cannot be avoided if a *true* edge as well as a sharp one is desired, and hence our advice to use the hone for nothing but section knives. Accuracy in a penknife or a razor for shaving is of no consequence; in a section knife it is a *sine qua non*.

The surface of the hone must not only be true and smooth, it must also be clean and free from dust and grit, a single particle of which may spoil the work of hours. It should therefore, when not in use, be kept constantly covered. It is always used with oil, and this oil should be of a kind that does not readily dry up. Good sperm is excellent, and so is purified neat's-foot oil. Avoid kerosene, soap and water, and similar fluids, which are so frequently recommended, especially under the guise of new discoveries. They have all been tried and found wanting. After use, the stone should be carefully wiped

clean, fresh oil being applied when it is next used. It must never be used without a liberal supply of oil, otherwise it will become glazed and will no longer act on the steel.

The hone being in good order, the blade is laid flat on it and moved over its surface with circular sweeps. The method of doing this is easily acquired, and is best learned by watching an expert cutler at work. If we could only hit it, the perfection of this operation would be to wear down the sides of the blade until they just met; they would then form a keen and perfect edge. Unfortunately we cannot always hit this point exactly; we are apt to overdo the matter just a little, and then comes up the *bête noir* of beginners—the “wire edge.” This is caused by the metal of the blade bending away from the hone as soon as it becomes of a certain degree of thinness. Once it does this it cannot be worn off by the hone, and the only way to remove it is to draw the edge of the knife or razor across a piece of horn or hard wood. After removing the wire edge in this way, give the blade one or two sweeps on the hone and then strop it. If the blade be very soft, the wire edge comes very quickly and easily, and is very hard to get rid of. On very hard blades the wire edge is not so apt to make its appearance. It comes, however, on very excellent blades. It may be avoided in a measure by driving the blade edge-first over the hone, and indeed the blade should always be sharpened most in this way. And since the backward and forward strokes are always nearly equal, as regards space passed over, this is accomplished by exerting a greater pressure on the forward than on the backward stroke.

This wire edge is a nasty thing when it breaks off on the hone. Unless removed it will very speedily ruin a fine knife, therefore look out for it and wipe off the hone carefully if you have any suspicion that particles of steel have broken off the blade and got on to it.

In honing, as in everything else, however, nothing but practice will impart skill, and he who intends to become an expert should practice on a few old razors, grinding, honing

and stropping them himself, until he has acquired the art of giving them an edge far keener than most of the razors ordinarily used for shaving.

The last operation to which the knife is subjected is that of stropping, and as the blade should be stropped after every section, it becomes important that our tools should be good and that we should know how to use them. By having two or three knives we can always avoid grinding and honing the blades ourselves, for it is then easy to hire the work done, and half-a-dozen knives will last a careful worker a long time with merely the aid of a good strop. But the strop he must use himself. Fortunately no great skill is required in stropping, but the strop must be a good one and is best home-made.

Our readers have doubtless seen the "Cheap John" strop-vendors take a rough table knife, strop it on one of their "patent" strops and cut a hair with it. Nevertheless such a knife, sharpened in that way, would not cut good sections, and such a strop is not just the thing for giving the finishing touch to a section knife, even though the label does say that it is "intended for surgical instruments."*

The strop should never be used with the intention of removing metal so as to thin the edge of the knife. This is the proper function of the hone, and those who depend most on the hone and least on the strop, will always succeed best. The strop is used in the first place for *smoothing* off the edge, and in the second for removing that incipient dullness which always comes from even the slightest use. It is true that in the latter case the strop trespasses somewhat on the functions of the hone, but only to a very slight extent, and stropping should never be carried so far as to require more than a very few strokes.

* The finest emery paper, glued on a strip of wood and used as a strop, will impart to a razor an edge sufficiently keen to enable it to remove the beard, and if paper three or four degrees coarser be used, a very dull knife or razor may be sharpened, and afterwards stropped on the finer emery until it is keen enough to cut a hair. But it will be found that cutting edges produced in this way are not smooth enough for section-cutting.

The best material for a strop is good calf-skin, well tanned, and firmly glued to a strip of wood with the hair side out. The leather should be hard, otherwise it will rapidly round the edge

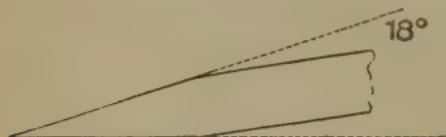


Fig. 11.

the edge of the blade as the latter passes over it, so that instead of two plane facets meeting at an angle of from 15° to 20° as left from the hone, (Fig. 12) the edge has become considerably

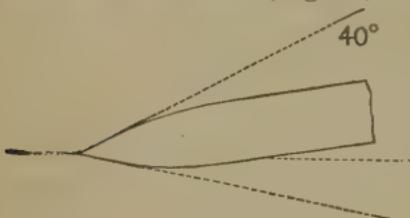


Fig. 12.

rounded and the ultimate angle of the cutting edge is nearly or quite doubled. The hardest and firmest leather should therefore be chosen, avoiding of course any leather that has been made hard and unpliant

by the action of water or other agents. Two sides will be found enough for a strop. On one the leather should be kept clean, while the other should be thoroughly impregnated with fine grained, but very hard rouge or crocus.* Rouge is an artificial oxide of iron prepared by exposing sulphate of iron to heat. The hardness of the resulting powder depends upon the temperature to which it has been exposed, and this temperature is very well indicated by the color which the rouge assumes. Bright red or crimson rouge is soft and will not cut steel; hard rouge, suitable for polishing steel, is purple in color, and this quality should therefore be chosen. Great care should be taken to see that it is free from gritty particles, and it should be well rubbed into the leather in a dry state. All mixtures of grease and oil with abrasive substances should be eschewed. If the leather be of good texture and the rouge hard and fine, a very

* Rouge may be purchased from dealers in watch-makers' tools. Those who cannot procure it readily may very easily prepare it for themselves. Full directions may be found in the "Amateur's Handbook." New York: Industrial Publication Company.

few strokes will suffice to impart the last degree of smoothness and keenness to the blade.

Since grit and dust would soon spoil the strop, it should be kept carefully covered and protected. The rouge will require occasional renewing, which may be done by sprinkling a little lightly over the surface and rubbing it in with the finger. Consequently we find that the best and most convenient holder for rouge is a bottle with some fine but porous fabric tied over the mouth of it, forming as it were a sort of dredge. Over the bottle mouth and its cover should be tied a paper cap to keep out dust.

We have thus endeavored to give, in as simple a manner as possible, such information as will guide the microscopist to the acquisition of skill in this most important but generally despised department of his art. We are told by the biographer of Swammerdam that a great deal of his success was to be attributed to his skill in sharpening his fine knives and scissors. The same is also stated of other noted workers, and yet none give even the slightest directions to the novice who desires to follow in their footsteps. There is not to our knowledge a single book in the English language which treats fully of this subject, with perhaps the exception of Holtzapffel's work on "Mechanical Manipulation," and this has long been out of print. As previously stated, however, it is only by practice that the necessary finger-skill can be acquired, but this skill is well worth acquiring at any cost. The man who depends upon cutlers and knife grinders will never make satisfactory progress.

NOTE H.

We are inclined to believe that the difficulty of enclosing glycerine does not arise from the dissolving action of this liquid, so much as from the great adhesiveness which exists between it and glass. Oily cements are of course attacked by glycerine, but shellac and several other gums are insoluble, and drying oils, if well oxidized are also insoluble. Where cells are used

they must be first firmly attached to the clean slide, and the cement should also be brought into direct contact with the clean cover. Glycerine does not evaporate at ordinary temperatures, and consequently wherever the slide or cover is soiled with it, no cement will ever adhere until the glycerine has been wiped off. There is no prospect of its drying.

NOTE I. Page 48.

The solution here referred to, and which is known as Klein-enberg's solution, is made in the following way:

(1.) Make a saturated solution of crystallized calcium chloride in 70 per cent. alcohol, and add alum to saturation. (2.) Make also a saturated solution of alum in 70 per cent. alcohol. Add (1) to (2) in the proportion of 1 : 8. To the mixture add a few drops of a saturated solution of hæmatoxylin in absolute alcohol.

This solution may be used in very many cases for staining sections, in place of the ordinary watery solution of logwood alum. It may, if required, be diluted with the mixture of 1 and 2. The stained sections are placed at once in strong spirit.

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Special Notice.

As our journal is too small and too low-priced to claim the attention of news dealers, we are compelled to rely almost wholly upon subscriptions sent directly to this office. As many persons would no doubt like to examine a few numbers before becoming regular subscribers, we will send four current numbers as a trial trip free.

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THE AMERICAN
JOURNAL OF MICROSCOPY,
AND
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